# THE INFLUENCE OF LEGUME CROPPING SEQUENCES ON ABOVEGROUND AND BELOWGROUND CARBON AND NITROGEN INPUTS IN PULSE CROP ROTATIONS

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By

Kathleen Slater

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## ABSTRACT

Pulse crops grown in prairie crop rotations can provide greater carbon (C) inputs than non-pulse crops in rotation and reduce nitrogen (N) fertilizer requirements. The aim of this research was to estimate the aboveground (ABG) and belowground (BG) partitioning of C and N inputs to soil from continuous (three year) chickpea (CP), lentil (L) and pea (P) systems and from CP, L and P grown in rotation with mustard (M) or wheat (W). Stable isotope techniques were used to label plants grown in a greenhouse and track residue C and N inputs to the bulk soil, heavy fraction organic matter (HF), light fraction organic matter (LF), very light fraction organic matter (VLF), water extractable organic matter (WEOM), the soil microbial biomass (SMB) and the inorganic N pool. Repeat-pulse $^{13}CO_2$-labeling and shoot $^{15}N$-labeling techniques revealed rhizodeposition of C and N was higher in non-continuous pulse crop systems (P-M-CP, P-W-CP, CP-W-CP, L-W-L, P-M-P and P-W-P), than in continuous CP, L and P. Belowground residue (roots and rhizodeposits) C made up 35%, 30% and 33% of total residue C in the continuous CP, L and P, respectively. Belowground residue C made up 50%, 43% and 25% of total residue C in CP, L and P in rotation with M or W, respectively. Belowground-N made up a greater proportion of total residue N than ABG-N in the continuous CP (56%), L (53%) and P (68%) systems, and in the non-continuous CP (76%), L (70%) and P (62%) rotations. Soil pool C and N did not differ between continuous CP, L or P, nor did it differ between the non-continuous CP, L or P rotations. There were no differences between M and W, as the ABG and BG residue C and N in the M pulse crop rotations did not differ from that of the W pulse crop rotations. There was a greater amount of C derived from rhizodeposition (CdfR) and N derived from rhizodeposition (NdfR) in the bulk soil and in the very light fraction organic matter (VLF) of the non-continuous pulse crop rotations, than in the continuous pulse crop systems. This research demonstrates the importance of BG inputs of C and N to soils from CP, L and P grown in rotation with M and W.

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| | |
|---|---|
| ABG | Aboveground |
| AMF | Arbuscular mycorrhizal fungi |
| BG | Belowground |
| BNF | Biological nitrogen fixation |
| CDC | Crop development centre, University of Saskatchewan |
| CP | Chickpea |
| %CdfR | Percentage carbon derived from rhizodeposition |
| DOM | Dissolved organic matter |
| GHG | Greenhouse gas |
| HF | Heavy fraction organic matter |
| IRMS | Isotope-ratio mass spectrometry |
| L | Lentil |
| LF | Light fraction organic matter |
| M | Mustard |
| NA | Natural abundance |
| %NdfR | Percentage nitrogen derived from rhizodeposition |
| P | Pea |
| SMB | Soil microbial biomass |
| SOC | Soil organic carbon |
| SOM | Soil organic matter |
| SON | Soil organic nitrogen |
| VLF | Very light fraction organic matter |
| W | Wheat |
| WEOC | Water extractable organic carbon |
| WEOM | Water extractable organic matter |

# 1. GENERAL INTRODUCTION

## 1.1 Introduction

Under the right conditions, the inclusion of pulse crops in cereal and oilseed crop rotations can provide environmental and economic benefits such as reduced greenhouse gas (GHG) emissions and a reduced dependence on synthetic nitrogen (N) fertilizers (Van Kessel and Hartley, 2000; Lemke et al., 2007; Nemecek et al., 2008; Statistics Canada, 2011). Wheat and canola are the dominant crops in Canada as they make up close to 50% of field crop area. Pulse crop production in Canada has been increasing since the 1980's and currently makes up 6% of field crop area or 2.2 million ha. Saskatchewan makes up the largest pulse production area in Canada, with 1.7 million ha., or 77% of total pulse crop area in Canada. Saskatchewan holds 87% of total chickpea (*Cicer arietinum*) area in Canada, 96% of total lentil (*Lens culinaris*) area in Canada and 68% of total pea (*Pisum sativum*) area in Canada (Statistics Canada, 2011). Increased emissions of GHG nitrous oxide ($N_2O$), methane ($CH_4$) and carbon dioxide ($CO_2$) have been associated with the increased use of N based fertilizers (Cole et al., 1997). Soil pool C and N dynamics are critical to better understanding global C-stores and sinks and thus relate to better understanding GHG emission dynamics. The potential for semi-arid cropping systems to sequester C is a more attainable goal with improved C and N budgets (Lal, 2004). The more precise nutrient budgets can be used to determine the best pulse crop rotations to reduce farmer dependency on N fertilizers and therefore improve environmental and economic sustainability of the system. Producers crop choices are typically influenced by market conditions and despite potential short term benefits from monoculture cropping, crop rotations typically decrease input costs and improve long term yields, resulting in improved returns (Meyer-Aurich et al., 2006; Livingston et al., 2012).

Growing pulse crops in rotation with non-pulse crops has become increasingly common in Canada, with 98% of farms with pulse crops reporting including them in rotation with other crops (Statistics Canada, 2011). Pulse crop rotations have shown positive impacts on soil nutrient cycling and crop yields due to contributions of bioavailable carbon (C) and N to soils (Van Eerd et al., 2014). Soil C inputs from pulse crops are derived from high quality aboveground (ABG) crop residue inputs, and belowground (BG) root exudates (Lemke et al., 2007; Wichern et al., 2007a,b). Symbiotic relationships formed with Rhizobia by pulse crops allow the plants to fix

atmospheric $N_2$, which contributes to higher N concentrations in roots and ABG residues (Soon and Arshad 2002; Gan et al. 2010). Nitrogen, as well as C can be released to the soil via the roots. The release of organic compounds, including cells, ions, root mucilage, exudates, secretes and fragments from living plant roots during growth is a process known as rhizodeposition (Nguyen, 2003; Wichern et al., 2008; Wichern et al., 2011). Carbon derived from rhizodeposition (CdfR) and N derived from rhizodeposition (NdfR) have been reported as 30% and 36% of total plant C and N, respectively, in pea (Wichern et al., 2007a). Furthermore, pulse crops in symbiosis with rhizobia can improve the environmental sustainability of cropping systems and crop yields through improved soil structure and interrupting cereal pest and disease cycles and increased soil microbial activity (Wichern et al., 2007a; Statistics Canada, 2011; Soudzilovskaia et al., 2012).

Providing a budget of both the plant and the root derived C and N in specific soil pools, such as the heavy, light and very light fraction organic matter, the water extractable organic matter, the soil microbial biomass pool and the inorganic N pool is possible through plant labeling with the stable isotopes $^{13}C$ and $^{15}N$ (Wichern et al., 2007 a, b; Comeau et al., 2013). The cotton stem wick technique delivers a constant supply of $^{15}N$ to plants and permits quantification of the total root derived N throughout plant growth (Russell and Fillery, 1996). Atmospheric repeat pulse labeling of plants with $^{13}CO_2$, in hermetic chambers allows the total root derived C added to the soil throughout plant growth to be quantified (Sangster et al., 2010). Further investigation of the simultaneous above and belowground inputs of C and N to soils from cereal-pulse crop rotations is required to better the understand C and N cycling dynamics and which pulse crop sequences can maximize C and N inputs and minimize N fertilizer use (Wichern et al., 2008).

The aim of this project was to determine the ABG and BG (roots and rhizodeposits) partitioning of C and N inputs to soil from chickpea, lentil and pea grown continuously and grown in rotation with mustard or wheat, using $^{15}N$ and $^{13}C$ stable isotope techniques. The distribution of the ABG and BG-C and N residues to plant parts and soil pools was also evaluated.

## 1.2 Organization of the Thesis

This thesis has been prepared in a manuscript format. This introduction and the literature review in Chapter 2 are succeeded by two research studies presented in Chapter 3 and Chapter 4.

The aim of the study in Chapter 3 was to determine the aboveground and belowground partitioning of C and N in continuously (three year) grown chickpea, lentil and pea. The pulse crops were grown under controlled conditions in a greenhouse and enriched with $^{13}C$ and $^{15}N$ in order to quantify the amount of C and N rhizodeposited to the soil.

Similarly to Chapter 3, Chapter 4 has the goal of determining the aboveground and belowground partitioning of C and N in chickpea lentil and pea; however, in this study a non-pulse crop (mustard or wheat) has preceded the pulse crops. The goal of Chapter 4 was to determine the ideal pulse crop rotation to maximize C and N residue returns to the soil. The pulse crops were grown under controlled conditions in a greenhouse and enriched with $^{13}C$ and $^{15}N$ in order to quantify the amount of C and N rhizodeposited to the soil. A synthesis of the primary findings of the research studies in Chapter 3 and Chapter 4 is presented in Chapter 5.

# 2. LITERATURE REVIEW

## 2.1 Pulse Crops

Pulse crops belong to the legume family and produce high protein seed known as "pulses" (Pulse Canada, 2015). Canadian pulse crop production yielded 5.7 million tonnes in 2010, with chickpea (*Cicer arietinum*), lentil (*Lens culinaris*) and pea (*Pisum sativum*) being the most commonly produced leguminous crops in the country. The majority of global chickpea, lentil and pea production takes place in Saskatchewan and occupies approximately 130,000 ha, 520,000 ha and 1.3 million ha, respectively (Agriculture and Agri-Food Canada, 2009).

Symbiotic relationships with *Rhizobacterium* and pulse crops result in biological fixation of atmospheric $N_2$ (BNF) which supplies N to the plant and to the soil. The decomposition of crop residues from ABG and BG sources also provides soil N inputs. Even when the contribution of N from a legume intercropped with cereals is minimal, the contribution is in most cases still greater than that of a non-leguminous crop (Peoples and Craswell, 1992). Pulse crop species, growth stage, species density within the crop mixture and soil management practices are factors that determine for the potential for pulse crops in crop rotations to fix $N_2$ from the atmosphere. Beyond contributing to soil N stores, pulse crops can improve the environmental sustainability of cropping systems and crop yields through improved soil structure, interrupting cereal pest and disease cycles and increased soil microbial activity (Wichern et al., 2007; Soudzilovskaia et al., 2012).

Nitrogen sparing occurs when a pulse crop takes up less N than would be required for a non-pulse crop, due to pulse crop N requirements being met via BNF. Nitrogen sparing along with release of mineral N from pulse crop residues can build soil $NO_3$ and can supply $NO_3$ to subsequent cereal crops (Herridge et al., 1995). Miller et al. (2002) reported a 21% increase in wheat yields when grown on pulse crop stubble, relative to yields grown on wheat stubble. Walley et al. (2007) reported that chickpea fix lower levels of N, causing them to create a soil N deficit or neutrality; however, with high water supply, chickpea had higher rates of N mineralization relative to canola, lentil, pea, wheat and mustard in a two-year study (Gan et al., 2009). Positive soil N balances following pulse crop growth do not always result in improved crop yields as the availability of N to crops following pulse crops is primarily dependent upon the rate of N mineralization (Jensen, 1993).

Due to the N additions to soils, including pulse crops in crop rotation with non pulse crops can result in a reduced dependence on synthetic N fertilizers, which could help to reduce GHG emissions produced by agriculture (Crews and Peoples, 2004; Nemecek et al., 2008). Lentil and pea are capable of levels of N fixation that create a N credit rather than depleting the soil (Crews and Peoples, 2004).

### 2.1.1 Pulse crop sequencing effects on soil carbon and nitrogen

Further research is required investigating the dynamics in which soil organic matter (SOM) pools are accumulating C and N in order to determine the ability for legume cropping systems to act as C sinks and improve their GHG budgets (Sollins et al., 2007; Fornara and Tilman, 2008; Flynn et al., 2012; Stockmann et al., 2013). Increases in WEOC where pulse crops have been added to a rotation with non-pulse crops have been reported but the processes controlling the increases in WEOC are unclear (Chantigny et al., 2003). The difference may be a result of distinct root exudation patterns by different crops.

The quality and quantity of plant litter added to the soil strongly controls the dissolved organic matter concentrations, and therefore the dissolved organic C and dissolved organic N concentrations of soils (Kalbitz et al., 2000; Puttaso et al., 2011). The amount of crop residues returned to soils is not always proportional to SOC content. Aboveground pulse crop residues typically have C:N ratios of 25:1 to 40:1, and cereal residues have C:N ratios of 70:1 to 100:1 (Stevenson and van Kessel, 1996). Amounts of aboveground plant residues are often 1.2 to 2 times less than wheat; however, the lower C:N ratio of legume residues can maintain equal or greater SOC levels than cereals (Lemke et al., 2007).

Crop residue quality, along with soil properties and environmental conditions will affect SOC levels as well. Legumes produce high quality crop residues, due to their lower C:N ratio and lignin content. Quick mineralization of legume crop residues is possible by a greater number of microbial taxa, relative to cereal crop residues (Brady and Weil, 2008). Both the quality and quantity of crop residues are important controls over SOC levels and must be considered when evaluating soil nutrient status and the sustainability of agricultural systems (Puttaso et al., 2011). A meta-analysis of crop rotation effects on soil C and N highlights the importance of using legumes as cover crops to build soil C and N across a wide range of rotations, soils and climates (McDaniel et al., 2014).

Crop rotations with greater diversity due to the inclusion of legumes and cereals, display enhanced nutrient cycling and availability (Liebig et al., 2006). A study examining the effects of crop sequencing and fertilization on soil properties in the western Corn Belt demonstrated that crop sequence had an effect on soil properties. A corn, soybean, sorghum and legume rotation showed the greatest improvements in nutrient cycling efficiency, relative to conventional cropping systems with no legumes (Liebig et al., 2002). Modeling the long-term impact of crop management on C sequestration in a Brown Chernozem at Swift Current, SK, revealed that the SOC level and sequestration rates were highest in cropping systems that included rye or pulse crops in rotation and that were well fertilized and frequently cropped (Congreves et al., 2015).

A short-term study found no significant cropping sequence effects on the organic C and total N content of soils cropped with two varying sequences of wheat, soybean and an annual legume over four years (Hao et al., 2001). Comparably, soil $NO_3$ levels were not affected by pea as a break crop in a three-year wheat rotation, relative to a canola-wheat rotation (Arshad et al., 2002). Conversely, there was an increase in soil N (0 to 120 cm) after harvest following pulse crops, relative to non-pulse crops, in Swift Current, SK (Miller et al., 2003). Sainju (2014) reported that a no-till barley-pea rotation with 40 kg N $ha^{-1}$ improved SOM and crop yields, relative to no-till continuous barley, no-till barley-fallow and conventional-till barley-fallow. In the Mediterranean climate of southern France, wheat intercropped with chickpea and wheat intercropped with lentil resulted in an increase in soil microbial biomass (SMB)-C relative to a continuous wheat rotation. The increased SMB-C was caused by an increase in the diversity of rhizodeposits introduced to the soil when a new plant species is introduced to the soil (Tang et al., 2014). The varying results on the concentrations of C and N indicate the need for further investigation into the role of legumes and crop sequencing on the input and fate of soil C and N.

## 2.2 Soil Organic Matter: C and N Cycling

### 2.2.1 Soil organic matter

Physical and chemical soil properties, such as soil structure, porosity, nutrient retention and cation exchange capacity, are enhanced by SOM. Maintaining a sustainable agricultural system is dependent upon providing sufficient SOM levels (Torbert et al., 1998; Janzen, 2006). The majority of SOM is stable and is composed of humic substances high in recalcitrant C,

which remains unchanged over longer periods of time relative to the non-humic substances. The stable SOM, or humus, displays fewer effects caused by land management changes, relative to the more labile portions of SOM (Sparks, 2003; Johnson et al., 2006). Stable SOM comes from the humification and decomposition of faunal and plant matter and further decomposition of the stable SOM produces the more labile SOM pool (Johnson et al., 2006). When greater concentrations of alleopathic molecules, such as alkaloids are present in the SOM, decomposition by microorganisms can decrease (Franzluebbers and Hill, 2005).

Photosynthetically derived C that is not respired by plants is harvested with the plant or it is added to soils as crop residue (Janzen et al., 1998). When the C added to soils from crop residues is humified at a rate greater than which the existing SOM is decomposed, SOM pools grow. The added crop residue C that is not humified is respired by the SMB, therefore, greater additions of more recalcitrant crop residues can result in greater accumulation of SOM (Ellert and Bettany, 1995; Janzen, 2006). The mineralization of SOM is strongly controlled by soil pH, texture, clay content and the C:N ratio (Brady and Weil, 2008). The products of SOM mineralization are ammonium ($NH_4$), nitrate ($NO_3$), $CO_2$ and methane ($CH_4$); however, certain bacteria such as nitrifying bacteria are able to utilize soil $CO_2$ and ammonia ($NH_3$) for growth and energy (De Boer and Kowalchuk, 2001).

### 2.2.2 Carbon and nitrogen cycling

The soil C and soil N cycles are coupled. The availability of soil N will affect the amount of C immobilized as new biomass, which ultimately affects the quantity and quality of SOM pools (Högberg, 2007; Thornton et al., 2007; Knicker, 2011). Likewise, the availability of C controls the ability of the SMB to remobilize and remineralize N. The dependence of N availability in soils upon C affects nutrient availability, SMB productivity, $CO_2$ production and denitrification (Saetre and Stark, 2005).

Inputs from crop residues and BNF are the main pathways that C and N respectively, enter soil systems. The differences in the quality and quantity of crop residue composition and nutrient availabilities from different crops affect nutrient cycles in different ways (Sparling et al., 1995; Paterson et al., 2003). A higher soil C:N ratio from greater inputs of crop residues with higher C:N ratios, causes greater immobilization and less mineralization of N. The addition of crop residues with lower C:N ratios, such as those of legumes, will increase rates of soil N

mineralization and nitrification, and reduce immobilization (Powlson, 1993). A lower soil inorganic N content will reduce plant growth and photosynthesis; however, there is a need to investigate the contribution of soil organic N (SON) to plant uptake and use of dual isotopes to improve knowledge of C and N interactions and their role in quantifying GHG emissions has been suggested (Gärdenäs et al., 2011). Alternative management practices such as combining legume crop rotations with livestock production have been suggested to reduce the uncoupling of the C and N cycles by livestock caused by the return of digestible N in urine and the release of digestible C to the atmosphere as $CO_2$ and $CH_4$ (Soussana and Lemaire, 2014).

**2.3 Soil Microbial Biomass**

Soil microbial biomass is a living component of SOM that is critical to soil productivity in all soil systems (Mäder et al., 2002). The SMB provides a labile source of C, N, P and S for plant uptake and plays an important role in soil formation and aggregation. Despite making up a small fraction of the SOM (approximately 1 to 4%), changes in SMB size and structure will impact soil fertility (Sparling, 1992; Mäder et al., 2002).

The soil microbial biomass is a biomarker of soil quality and metabolic status. There is quick feedback between soil disturbance and SMB-C and SMB-N cycling (Salínas-Garcia et al., 1997). The SMB-C and SMB-N pools are very active and rapid responding components of SOM, therefore changes in these pools can be indicators for subsequent changes in SOM occurring at slower rates (Körschens et al., 1998).

The presence and activity of SMB is affected by crop rotation, since the quantity and quality of C and N additions to soils are plant species specific (Nikièma et al., 2012). The SMB-C:SMB-N ratio is as an indicator of whether the SMB community is dominated by bacteria or fungi. Ratios of 7:1 to 12:1 have been linked to communities dominated by fungi and a ratio of 3 to 6 is associated with bacterial dominance in soils (Moore et al., 2000). Breulmann et al. (2012) reported changes in SMB communities caused by the presence of legume species in semi-natural grassland systems. Furthermore, Breulmann et al. (2012) suggest that with fungal dominance in soils there is more efficient nutrient cycling and greater SOC accumulation. A bacterial-dominated system is associated with more rapid rates of decomposition and nutrient cycling.

Leguminous species have been introduced for the conversion of agricultural land to remediated grasslands, on the Loess Plateau (Li et al., 2012). Two of the legume species

(*Astragalus adsurgens* & *Medicago sativa*) investigated improved the SOC, SON and SMB pools after three years, and even more so after five years. A third legume species (*Melilotus suavolens*) did not affect any of the measured soil parameters after three years and caused negative impacts after five years. The negative impacts were attributed to soil moisture depletion caused by the plants' high water demands. The soil quality improvements by *A. adsurgens* and *M. sativa* were a result of improved plant biomass causing greater OM inputs to the soil that were rich in available N and allowed SMB to prosper. Higher SMB-C and SMB-N pools in the Loess Plateau land conversion suggests that the SMB was dominated by fungi, which has been reported to result from the introduction of leguminous species in previous studies, as well as in the Li et al. (2012) study (Kara and Bölat, 2008; Breulmann et al., 2012). Wardle et al. (1992) report a range of 0.3 to 4% SMB-C in the topsoil of agricultural soils. In the top 15 cm of agricultural soils in Alberta, SMB-C ranged from 1.9 to 3.3% in conventional till and 1.3 to 3.8% under zero till (Carter and Rennie, 1982).

Chang and Juma (1996) concluded that crop rotations that include legumes affect the soil biological properties and N availability after finding greater SMB-N beneath a barley-faba bean rotation than under continuous barley. Root residues from the previous year contributed to greater SMB-C and SMB-N levels, the following spring. Carboxylic acids and sugars are the primary C substrates utilized by the SMB beneath legume species. These labile C substrates are likely a result of the tendency for legumes to provide immediately available substrates via root exudation (Nanniperi et al., 2008). A meta-analysis of crop rotation effects on soil C and N found SMB-C and SMB-N increased by 20.7% and 26.1%, respectively, when a leguminous cover crop was added to a monoculture (McDaniel et al., 2014). Studies investigating the effects of leguminous and non-leguminous crop rotations on SMB-C and SMB-N cycling are required to provide new data across a wider range of leguminous crop rotations.

### 2.3.1 Chloroform fumigation extraction

The fumigation extraction method quantifies the SMB pool of soils and allows characterization and estimation of SMB-C and SMB-N. Soils are fumigated with chloroform ($CHCl_3$) for 24 hours and then $NH_4$-N, total N and total SOC are extracted using 0.5M $K_2SO_4$. Fumigation causes greater SMB cell lysis, which increases the amount of C and N extracted from the pool (Brookes et al., 1985).

### 2.3.2 Calculating the soil microbial biomass extracted C and N

The amount of extracted C of N is calculated following Vance et al. (1987):

$$SMB_{C/N}=K_{EC/N}X\ E_{C/N}$$

Where $E_{C/N}$=(SOC/SON extracted with fumigation)-(SOC/SON extracted without fumigation)

and $K_{EC}$ is 0.36 (Joergensen, 1996; Vance et al., 1987) and the $K_{EN}$ is 0.54 (Joergensen and Mueller, 1996; Brookes et al., 1985).

## 2.4 Rhizodeposition

Rhizodeposition is defined by Wichern et al. (2008) as the release of inorganic and organic complexes from live roots. All materials left in the soil excluding the roots, but including sloughed tissues and hairs, and mucilage as well as vitamins, organic acids, hormones, amino acids, sugars and ions are considered rhizodeposits (Jensen, 1993). Breulmann et al. (2012) suggest that rhizodeposits are more readily available to the SMB than older more recalcitrant SOM. Rhizodeposits and roots contribute an important amount of C and N to soils and if unaccounted for, soil nutrient budgets are underestimated (Walley et al., 2007). There is a rhizosphere-priming effect on SOM decomposers that arises from root exudates, which directly influences C and N cycling and also may impede nitrification by nitrifying microorganisms (Haichar et al., 2014).

Studies investigating the simultaneous input of C and N from rhizodeposition are required since separate C and N studies have shown that a significant proportion of these essential and coupled nutrients is derived from belowground deposits (Wichern et al., 2007; Sangster et al., 2010).

### 2.4.1 Rhizodeposition of carbon

Carbon derived from rhizodeposition in mature oats accounts for 31% of total plant C and in mature pea it accounts for 30% of plant C (Wichern et al., 2008). The addition of C from plant roots to soils is available to the soil microorganisms and results in improved nutrient acquisition (Nguyen, 2003). Cereals transfer approximately 20% to 30% of absorbed C into soils (Kuzyakov and Domanski, 2000). Approximately 50% of this C is found in the plant roots and 30% is found

in $CO_2$ produced from the soil by microbial and root respiration. The remaining BG-C is incorporated in the SOM and the SMB.

Mercx et al. (1987) suggest that an elevated C:N ratio from rhizodeposited C can result in greater C availability and reduced N availability. Nitrogen becomes temporarily unavailable for plant uptake due to immobilization by the SMB. This demonstrates the role of competition between roots and SMB for N and also the control of root exudation on C and N availability. Similarly, Liljeroth et al. (1990) report that N fertilizers can decrease the below ground translocation of assimilated C in wheat plants. There is evidence that rhizodeposition of C is managed by plants in order to control SMB activity on N immobilization (Paterson et al., 2003).

### 2.4.2 Rhizodeposition of nitrogen

Plant roots release N in the form of $NO_3^-$ and $NH_4^+$ ions, as well as in sloughed root tissues, mucilage, amino acids and lysates (Uren, 2007). Rhizodeposition of N is generally higher in legumes than in cereals because of their $N_2$ fixing abilities in symbiosis with *Rhizobium* (Wichern et al., 2007a, b). In a review of rhizodeposition methods and estimates Wichern et al. (2008) reported a 16% median of NdfR in legumes and a 14% median in cereals. Arcand et al. (2013b) reported that BG-N (root N and rhizodeposited N) made up 61% of total residue N. The majority of rhizodeposited N is immobilized in microbial residues and contributes to a labile pool of SOM, which is the main source of rhizodeposited N for remineralization (Mayer et al., 2003).

### 2.4.3 Isotope methodologies for estimating carbon rhizodeposition

Carbon rhizodeposition can be quantified using C isotopes such as $^{14}C$, $^{13}C$ and $^{11}C$. The most effective methods for estimating BG-C rhizodeposition are continuous $^{13}C$ labeling or repeat pulse $^{13}C$ labeling (Kuzyakov and Domanski, 2000; Sangster et al., 2010). Continuous labeling provides an estimation of the total amount of C transferred from the plant to the soil and BG soil pools throughout the labeling period (Meharg, 1994). Continuous labeling requires specialized, expensive equipment that exposes plants to $^{13}CO_2$ over an extended period of time.

Atmospheric $^{13}C$ pulse labeling with $^{13}CO_2$ involves exposing plants to a $^{13}CO_2$-enriched environment over shorter time periods at specific growth stages or time intervals (Sangster et al., 2010). Pulse labeling is an easier and cheaper process than continuous labeling and can provide

information on the distribution of more recently produced photosynthates at specific growth periods. Recently fixed C compounds (exudates, root cell lysis, sloughed root cells and associated polysaccharides) dominate the recovered C in pulse labeling experiments. The products of $^{13}CO_2$ pulse labeling display the relative distribution of C at the time of labeling (Kuzyakov and Domanski, 2000); however, the pulse labeling of pod-producing plants does not produce a homogenous distribution of $^{13}CO_2$ and therefore repeat pulse labeling is required with pod-producing plants. Sangster et al. (2010) have shown that CdfR additions to the SOM pools can be estimated with $^{13}CO_2$ repeat pulse labeling.

### 2.4.4 Isotope methodologies for estimating nitrogen rhizodeposition

Nitrogen rhizodeposition from plants is commonly estimated using $^{15}N$ plant labeling techniques (Wichern et al., 2008). Plants can be labeled with $^{15}N$ using shoot labeling, leaf labeling, root labeling and atmospheric labeling. Atmospheric labeling and the split root technique allow continuous labeling, which provides homogenous distribution of $^{15}N$ to plant parts and a representative estimate of the amount of N derived from rhizodeposition.

Pulse labeling, as opposed to continuous labeling, can be accomplished using shoot-labeling techniques. The distribution of $^{15}N$ after pulse labeling is not always representative of the total plant N distribution, due to heterogeneous distribution of the $^{15}N$ label (Sangster et al., 2010). Younger photosynthates tend to be more enriched than older ones when pulse labeling is used (Kuzyakov and Domanski, 2000). Isotopes from rhizodeposition are therefore not necessarily representative of the entire growth phase. Repeat pulse labeling, has shown potential in representing plant N distribution (Mayer et al., 2003; Wichern et al., 2008; Arcand et al., 2013a,b; Arcand et al., 2014).

The stem wick method is a $^{15}N$ shoot labeling technique that involves drilling a hole through the plant stem and passing a cotton thread through it (Russell and Fillery, 1996; Wichern et al., 2008). The ends of the thread are immersed in a sealed vial holding a $^{15}N$-urea solution. A silicone covering is placed over the thread to stop evaporation and plasticine is used to hold the thread and tubing taut against the plant stem. The $^{15}N$-urea solution can be replenished via injection through the vial cap, at regular intervals or as required.

Labeling can only occur once the stem is thick enough; therefore, early growth stages cannot be included in the labeling procedure (Wichern et al., 2008). Callus tissue can form where

the hole has been drilled resulting in reduced uptake of the $^{15}N$-urea solution. The vials require frequent monitoring to ensure solution uptake is not impeded (Russell and Fillery, 1996).

### 2.5 Characterization of Soil C and N

Density fractionation separates SOM into two physically and chemically separate portions known as the light fraction (LF) and the heavy fraction (HF) (Carter and Gregorich , 2008). The LF is more labile than the HF and considered a primary source of C and N in agricultural soils (Ford and Greenland, 1968). The HF is more humified, contains mineral-bound materials, and usually has a lower C:N ratio than the LF (Carter and Gregorich , 2008). The LF organic matter is extracted by suspension in sodium iodide (NaI), which has a specific gravity of 1.7 g $cm^{-1}$. The soil subsamples are mixed in NaI and upon settling, the LF floats at the surface of the solution.

Soil organic C (SOC) and N (SON) stores are commonly quantified by isolating the dissolved organic matter (DOM). Water extractable organic matter (WEOM) is included in the DOM category and consists of root exudates, plant residues and SMB. Dissolved organic matter is essential to soil processes as it functions as an intermediary for C fixation, a C sink and also facilitates amino acid transport for nitrification (Zolsnay, 1996). Microbial activity and mineralization processes cause a greater release of DOM into the soil solution (Kalbitz et al., 2000). Agricultural rotations that include legumes commonly produce greater amounts of water extractable organic carbon (WEOC) than non-leguminous rotations (Chantigny et al., 1997; Campbell et al., 2000; Comeau et al., 2013). Chantigny et al. (2003) suggest that the greater amount of exudates produced by the legumes, act as an indicator to *Rhizobia* to increase microbial activity, resulting in a greater production of WEOC.

Extracting the WEOM involves mixing soil samples with 5 mM calcium chloride ($CaCl_2$) for 10 minutes, at a 2:1 ratio (volume:dry mass equivalent) (Zolsnay, 1996). The extracts are then filtered through a vacuum filter unit with a 0.4 μm polycarbonate filter (Carter and Gregorich, 2008). The HF, LF and WEOM can be analyzed for C and N content by conversion to $CO_2$ and gas chromatography (Maynard et al., 1993).

### 2.5.1 Calculating total soil organic carbon, rhizodeposition and root biomass

The SOC or SON content of a particular soil pool (A), following Subedi et al. (2006), can be calculated as:

A= dry weight of soil pool (g) × (%SOC or SON/100)

The percentage of rhizodeposition (B) can be calculated after Janzen and Bruinsma (1989):

$$B = \%NdfR = \frac{(\text{atom}\%^{15}N - \text{atom}\%^{15}N \text{ background X})}{(\text{atom}\%^{15}N - \text{atom}\%^{15}N \text{ background Y})} \times 100$$

or

$$B = \%CdfR = \frac{(\text{atom}\%^{13}C - \text{atom}\%^{13}C \text{ background X})}{(\text{atom}\%^{13}C - \text{atom}\%^{13}C \text{ background Y})} \times 100$$

In the calculation above, X is the background $^{13}C$ or $^{15}N$ in the control soil, where plants that have not been labeled with $^{15}N$ and $^{13}C$ are grown; Y is the background $^{13}C$ or $^{15}N$ in the control roots of non-labeled plants.

The amount of C or N rhizodeposition (C) can be calculated by multiplying total C or N content in the soil pool of relevance with the %CdfR or %NdfR. In the SOC or SON pool, total rhizodeposited C is derived as:

$$C = A \times B/100$$

When calculating %NdfR and %CdfR it is assumed that $^{15}N$ and $^{13}C$ enrichment of the roots is equal to that of the rhizodeposits, and that the enrichment of the fine rootlets is equal to that of the roots. It is also assumed that the enrichment of the roots with $^{15}N$ and $^{13}C$ is constant throughout the experiment (Wichern et al., 2007).

# 3. THE INFLUENCE OF LEGUME CROPPING SEQUENCES ON ABOVE AND BELOWGROUND CARBON AND NITROGEN INPUTS IN CONTINUOUS CHICKPEA, LENTIL AND PEA

## 3.1 Abstract

A greater focus on environmental and economic sustainability in agricultural systems has led to an examination of the role of pulse crop rotations as a means to reduce synthetic N fertilizer use. However, the effects of increasing the frequency of pulse crop in rotation are not well understood. Furthermore, a better understanding of the effects of continuous pulse crop growth on the aboveground (ABG) and belowground (BG) partitioning of C and N is required. The aim of this study was to estimate the ABG and BG partitioning of C and N inputs to soil from continuous chickpea, lentil and pea systems, using $^{15}N$ and $^{13}C$ stable isotopes, and to determine the ABG and BG distribution of C and N residues in plant parts and soil pools. In chickpea, lentil and pea, the BG (roots and rhizodeposits) C was 35%, 30% and 33% of total residue C, respectively and did not differ between crop treatments ($P$>0.05). Belowground N made up 56%, 53% and 68% of total residue N (including roots and rhizodeposits) in chickpea, lentil and pea, respectively, and did not differ between crop treatments ($P$>0.05). The amount and distribution of C, $^{13}C$, N and $^{15}N$ did not vary between chickpea, lentil and pea in the bulk soil, heavy fraction organic matter (HF), light fraction organic matter (LF), very light fraction organic matter (VLF), soil microbial biomass (SMB) or inorganic N pools ($P$>0.05). The amount and distribution of CdfR and NdfR did not differ between chickpea, lentil and pea in any of the soil pools ($P$>0.05).

## 3.2 Introduction

In 2012, agriculture in Canada contributed 8% or 56 million tonnes of $CO_2$ equivalent emissions to Canada's total emissions inventory (Environment Canada, 2014). Globally, 87.2% of nitrous oxide ($N_2O$) emissions were derived from agriculture, primarily from agricultural soils and from animal wastes (Signor and Cerri, 2013). The high contribution of $N_2O$ to GHG emissions is of concern since the global warming potential of $N_2O$ is 300 times greater than the global warming potential of $CO_2$ (IPCC, 2006).

When symbiotic relationships with *Rhizobacterium* are formed, pulse crops can fix

atmospheric $N_2$ and provide a supply of mineralized N to the soil and the plant. Furthermore, their crop residues typically have lower C:N ratios (25:1 to 40:1), than cereals (70:1 to 100:1) which provides more readily available residues to the soil microbial biomass (SMB) for incorporation into soil SOM (Stevenson and van Kessel, 1996; Franzluebbers and Hill, 2005). These characteristics make leguminous pulse crops an attractive rotational crop for producers as they result in a reduced requirement for synthetic N fertilizers, which gives them the potential to reduce GHG emissions and yield financial savings (Crews and Peoples, 2004; Nemecek et al., 2008).

Beyond soil N benefits, legumes can improve soil quality and health status by reducing cereal pests and disease cycles, as well as by helping to manage weeds and soil fertility, particularly in organic systems (Moncada and Schaeffer, 2010). It has also been suggested that the quantity and quality of SOC under pea may be comparable to that of cereal crops due to similar yields of residue C inputs (Lemke et al., 2007). Sainju and Wang (2014) found increased SOC under a no till barley/pea rotation with fertilizer added to barley at 80 kg N $ha^{-1}$ in the Northern Great Plains. The greater amounts of residue added to the soil from barley and pea compared to other treatments, followed by the lower C:N ratio of the pea residues than in the barley residues likely improved the incorporation of residue C into SOC.

One of the processes by which pulse crops increase soil C and N, apart from ABG residue inputs, is rhizodeposition. The BG process of rhizodeposition occurs when live plant roots transfer organic and inorganic complexes to the soil (Wichern et al., 2008). Amongst the complexes transferred from the roots are sloughed root cells, tissues, mucilage and hairs along with organic acids, hormones, vitamins, ions and sugars (Jensen, 1993). A substantial amount of C and N is contributed to soils from roots and rhizodeposition and if unaccounted for, soil nutrient budgets are significantly underestimated (Walley et al., 2007).

Belowground N in pea has been reported as 61% of total plant residue N, with 11.5% of the BG-N in pea being from the roots and 49.1% being derived from rhizodeposition (NdfR) (Arcand et al., 2013b). It is crucial to consider C cycling in soils when investigating N dynamics, as the cycles are coupled. The quantity and quality of SOM in the soil is dependent upon the availability of N, which will affect soil C immobilization (Knicker, 2011).

Studies investigating the rhizodepostition of pea suggest that CdfR is more easily available to the soil microbial community and enhances nutrient acquirement compared to shoot

derived C (Nguyen, 2003; Wichern et al., 2007b). Rhizodeposition of C in mature pea plants has been estimated as 30% of total plant C, which is similar to oats, with CdfR making up 31% of total plant C (Wichern et al., 2007a). Fornara and Tilman (2008) reported 500% more soil C storage in a 12-year experiment where legumes were included in a perennial grassland site grown on degraded agricultural land. The improvements in N storage as well as C storage at this site were attributed to BNF by the legume crops; i.e., the increased availability of N for the grass crops increased root biomass, which ultimately improved SOC storage.

The differences in the above studies as well as the inherent link between C and N cycling and SOM storage, highlights the need for further research on the effects of continuous pulse crop growth as well as pulse crops grown in rotation with non-pulse crops and how the ABG and BG partitioning of C and N are affected. Lower arbuscular mycorrhizal fungi (AMF) colonization of roots has been reported in continuous pea systems, relative to pea in rotation with wheat; however, the differences in AMF colonization between the two pulse crop systems is unlikely to cause significant biological changes to the soil (Nayyar et al., 2009). Understanding the C and N partitioning in continuous pulse crop rotations is therefore important for comparative use to intercropping studies with pulse crops (Knight, 2012). Continuous rotation studies can be used as reference materials to future studies investigating pulse crops in rotation with cereals and oilseeds.

The objectives of this study were to (1) estimate the ABG and BG partitioning of C and N inputs to soil using $^{15}N$ and $^{13}C$ stable isotopes, (2) investigate C and N residue inputs of chickpea, lentil and pea into soils previously cropped with chickpea, lentil and pea and (3) determine the ABG and BG distribution of C and N residues in plant parts and soil pools.

## 3.3 Materials and Methods

### 3.3.1 Experimental design and planting

Soil cores were extracted from an Orthic Brown Chernozemic soil at the Agriculture and Agri-Food Canada Semiarid Prairie Agricultural Research Centre (SPARC) in Swift Current, Saskatchewan, Canada (50°15′N, 107°44′W) (Ayers et al., 1985). The soil cores were extracted in August 2011 and were 20 cm in diameter and 30 cm in depth. Table 3.1 shows the crop rotations grown in the field the cores were collected from. In 2012 the cores were cold stored and

frozen over the winter. The cores were dry until moved into the greenhouse for seeding.

**Table 3.1. Crop rotation history of the continuous pulse crop soil cores used in experiment one. Chickpea, lentil and pea were grown for the third year in a row in the university greenhouses in 2013.**

| Crop grown in SPARC field trial | | | Crop grown in greenhouse study |
|---|---|---|---|
| 2009 | 2010 | 2011 | Spring 2013 |
| Wheat | Pea | Pea | Pea |
| Wheat | Chickpea | Chickpea | Chickpea |
| Wheat | Lentil | Lentil | Lentil |

*Soil cores were taken at the end of this rotation phase
Number of cores = 3 treatments x 4 reps (enrichment) + 3 treatments x 4 reps (natural abundance) = **24 cores**

In the University of Saskatchewan greenhouses, the cores were arranged in a randomized complete block design. Eight pea (cv. CDC Meadow), chickpea (cv. CDC Cory) or lentil seeds (cv. CDC Imvincible CL) were planted in replicate (n=4) soil cores on March 21, 2013 and culled to four plants per pot following germination.

Immediately before seeding, peat-based inoculant containing *Rhizobium leguminosarum* bv. *viciae* (Nodulator®XL, Becker Underwood, Saskatoon, SK) was applied as directed by the manufacturer to all pea and lentil seeds. All chickpea seeds were treated with a peat-based inoculant for chickpea containing *Rhizobium leguminosarum* bv. *Bradyrhizobium* (Nodulator®XL, Becker Underwood, Saskatoon, SK). The chickpea, lentil and pea seeds were inoculated at five times the recommended rate. The plants were watered every other day and half of the cores (12) were kept isolated in a separate greenhouse to avoid contamination with $^{13}C$ and $^{15}N$. The isolated soil cores and plants were the natural abundance (NA) cores and plants. They were used as background values for calculations [Eq 3.1 & 3.2].

### 3.3.2 $^{15}N$ labeling

A $^{15}N$ -enriched urea solution was fed to the plants using the stem wick method (Russell and Fillery, 1996; with modifications by Mayer et al., 2003 and Mahieu et al., 2009) (Fig 3.1). The stem wick method involves drilling a 0.5 mm hole through the plant stem 5 cm above the soil. A thin sewing needle was used to pull a 10 cm cotton thread through the drilled hole. The thread was inserted through silicone tubing (0.76 mm i.d. × 4 cm length) to reduce evaporation of the $^{15}N$ solution. The tubing was held in place against the stem with plasticine. The other end

of the tubing and thread was placed into 0.35 mL 0.4 % (w/v) $^{15}$N enriched urea solution (98.2 atom% $^{15}$N), held in a 2 mL vial with a secured cap. The Teflon coated silicone septum in the cap had a space that allowed the tubing and thread to pass through it into the vial, with minimal solution losses via evaporation.

**Figure 3.1. Photograph of the stem wick method on chickpea plants grown in a greenhouse.**

A total of 2.5 mL $^{15}$N-urea solution applied to each plant in 0.20 to 0.35 mL increments, over five weeks. The solution was applied every two to five days. After the final application of $^{15}$N enriched urea solution was taken up by the plants, 0.40 mL of deionized water was injected into the vials to ensure complete uptake of the $^{15}$N-urea solution.

Due to development of a callus tissue where the stem was drilled, all plants were re-wicked at least once during the experiment with some plants being re-wicked two to three times throughout the labeling period. It was clear a callus had formed because uptake slowed significantly and the stem became very hard in the area where it had been pierced. A total of 4.9 mg $^{15}$N-urea was supplied to each plant from 26 to 61 days after sowing.

### 3.3.3 $^{13}$C labeling

The plants were labeled with $^{13}$C in airtight polymethyl methacrylate chambers, following Sangster et al. (2010). Each chamber accommodated four soil cores. The cores were exposed to a $^{13}$C-enriched atmosphere once per week for 20 to 60 minutes, depending on the rate of

photosynthesis at different growth stages. There were six weekly labeling events, which began and ended 26 and 61 days after sowing, respectively. During each labeling event, GLAD Press'n Seal Freezer® wrap (The Clorox Company, Oakland, CA) was used to isolate the soil from the $^{13}C$-enriched atmosphere. Covering the soil prevents dilution of the $^{13}C$ label via soil respiration.

The chambers were placed over each block of four cores and plants (Fig 3.2). Once the $CO_2$ level dropped to 66% of its original value, $^{13}CO_2$ gas was produced inside the chamber, to replace the removed $^{12}CO_2$, depleted through photosynthesis. The $CO_2$ concentration in the chambers during each labeling event was maintained at or near ambient levels (380 to 430 ppm). The $^{13}CO_2$ atmospheric enrichment was maintained at 33% during each labeling event. Fans were attached to the inside of the chambers to circulate the air during labeling events.

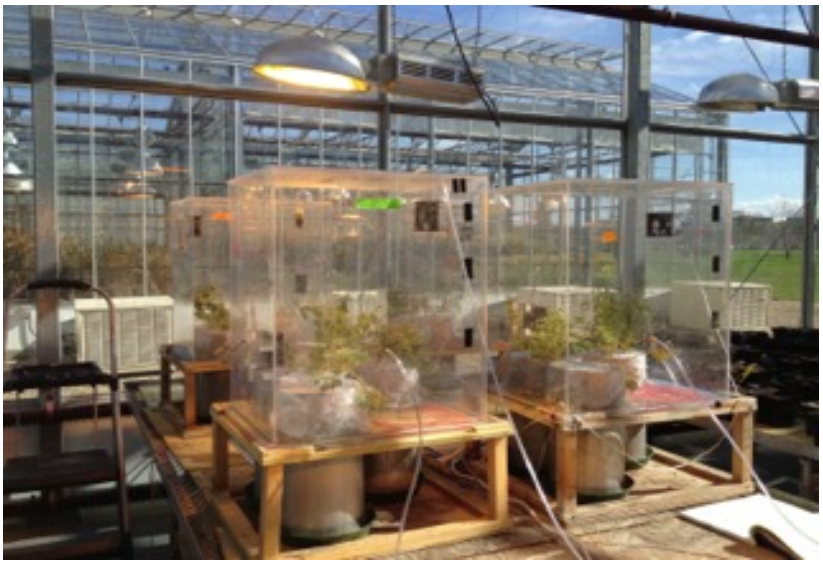

Figure 3.2. Photograph of experimental set up of soil cores and plants in the greenhouse with repeat-pulse $^{13}CO_2$ labeling chambers over plants.

The $^{13}CO_2$ was produced by injecting 4*M* hydrochloric acid (HCl) into a beaker suspended in the chamber that contained a solution of $^{13}C$-enriched sodium bicarbonate ($NaH^{13}CO_3$) (99 atom% $^{13}C$). The HCl was injected into the solution again once the initial $CO_2$ spike dropped. This was repeated until no more $CO_2$ was produced. Infrared gas analyzers (IRGA) (S151 Infrared $CO_2$ Analyzer, Qubit Systems, Kingston, Ontario) connected to each chamber, were used to observe the total $CO_2$ concentration in the chambers throughout labeling. A data logger (Logger Pro, Qubit Systems) recorded the concentrations every 20 s. Each chamber was exposed to a total of 303.4 mg $^{13}C$ during the six labeling events.

### 3.3.4 Plant and soil analysis

Labeling ceased at maturity; one week after labeling ceased the plants were harvested and separated into leaves, stems, pods and seed. Each soil core was dismantled and visible roots as well as fine roots were removed by hand and washed. The bulk soil and plant parts were dried for two days at 60°C and then ground using a coffee grinder followed by a ball mill. The plant and bulk soil subsamples were encapsulated and analyzed for %C, %N, and atom%$^{13}$C and atom%$^{15}$N, using a Costech Elemental Combustion System (Costech Analytical 191 Technologies, Inc.) paired with a Delta V Advantage Mass Spectrometer (Thermo Fisher 192 Scientific Inc.).

Soil $NO_3^-$-N and $NH_4^+$-N were extracted from each soil core using 2*M* KCl (Carter and Gregorich, 2008). The extracts were quantified colorimetrically with a SmartChemTM 2000 (Westco Scientific Instruments, Brookfield, CT). Enrichment of $^{15}$N in the $NO_3^-$ and $NH_4^+$ pools in the KCl extracts was determined by analyzing the $^{15}$N diffused onto filter paper disks acidified with 2.5*M* $KHSO_4$ and enclosed in polytetrafluoroethylene tape (Stark and Hart, 1996).

Following Gregorich and Beare (2007), the HF and LF organic matter pools were isolated using NaI, which has a density of 1.7 g $mL^{-1}$. The 50 g subsamples were mixed with 100 mL NaI for one hour and then left to stand at room temperature for 48 hours. The slurry was filtered through a vacuum filter unit with a 0.4 μm polycarbonate filter. The LF was further fractionated into VLF and LF, following the same procedure as for the HF but substituting deionized water for NaI, (density = 1.0 g $mL^{-1}$). The HF and LF subsamples were also dried, encapsulated and analyzed for %C, %N, and atom%$^{13}$C and atom%$^{15}$N, using the Costech Elemental Combustion System (Costech Analytical 191 Technologies, Inc.) paired with a Delta V Advantage Mass Spectrometer (Thermo Fisher 192 Scientific Inc.).

Soil microbial biomass was isolated using the chloroform fumigation method (Voroney et al., 2008). The soils were covered and incubated for 7 days at 50% water holding capacity, at 25°C. The soils were maintained at 50% water holding capacity throughout incubation. Three 50 g subsamples of incubated soil from each core were placed in an airtight desiccator and fumigated with ethanol-free chloroform for 24 hours. Three non-fumigated 50 g subsamples of soil were extracted with 100 mL 0.5*M* $K_2SO_4$ and filtered into vials. The fumigated soil subsamples were then extracted with 100 mL 0.5*M* $K_2SO_4$ and filtered into vials. The extracts were kept frozen until analysis. Enrichment of $^{15}$N and the concentration of N in the SMB

extracts were determined following the Stark and Hart (1996) methodology for the recovery of $^{15}N$ from persulfate digests. Enrichment of $^{13}C$ and the concentration of C in the SMB extracts was determined by IRMS analysis of the salt that remains in the vial after evaporation of the liquid extracts in a drying oven at 40°C.

### 3.3.5 Calculations

Nitrogen and C derived from rhizodeposition were calculated as (Janzen and Bruinsma, 1989):

$$B = \%NdfR = \frac{(atom\%^{15}N - atom\%^{15}N \text{ background X})}{(atom\%^{15}N - atom\%^{15}N \text{ background Y})} \times 100 \qquad [3.1]$$

or

$$B = \%CdfR = \frac{(atom\%^{13}C - atom\%^{13}C \text{ background X})}{(atom\%^{13}C - atom\%^{13}C \text{ background Y})} \times 100 \qquad [3.2]$$

where background-A is the atom% $^{13}C$ and $^{15}N$ values in the soil of plants grown under NA conditions, and background-B is the atom% $^{13}C$ and $^{15}N$ values in the roots of plants grown under NA conditions (Schmidtke, 2005). Individual %NdfR and %CdfR values were calculated for the bulk soil, the HF, LF and VLF soil pools, and the SMB pool. A %NdfR value was also calculated for the soil $NO_3^-$ and $NH_4^+$ pools. The amount of CdfR and NdfR (mg core$^{-1}$) in each soil pool was calculated by multiplying the %CdfR and %NdfR in a specific soil pool, by the total C and N (mg core$^{-1}$) in the respective soil pool. The rhizodeposition values are representative of net C and N rhizodeposition and do not consider reabsorption of C and N by the plant. Three assumptions made when using this calculation are that (1) there was a uniform distribution of $^{13}C$ and $^{15}N$ within the root system; (2) $^{13}C$ and $^{15}N$ enrichment was constant throughout growth; and (3) there was equal $^{13}C$ and $^{15}N$ enrichment of the recovered roots and rhizodeposits (Janzen and Bruinsma, 1989; Mayer et al., 2003).

The distributions of plant C, N, $^{13}C$ and $^{15}N$ were calculated as a percentage, by dividing the amount of C, N, $^{13}C$ or $^{15}N$ (mg core$^{-1}$) in a particular plant part or the rhizodeposits by the total amount of C, N, $^{13}C$ or $^{15}N$ (mg core$^{-1}$) in all of the plant parts and rhizodeposits, without

consideration of soil $^{13}C$ and $^{15}N$ (Russell and Fillery, 1996).

The distribution of C, N, $^{13}C$ and $^{15}N$ within the entire plant-soil system was calculated as a percentage by dividing the amount of C, N, $^{13}C$ or $^{15}N$ (mg core$^{-1}$) in a particular soil pool by the total amount of C, N, $^{13}C$ or $^{15}N$ (mg core$^{-1}$) in the bulk soil, as well as in all of the plant parts and rhizodeposits.

### 3.3.6 Statistical analysis

All statistical analyses were performed with the computer program R (version 2.15.2 GUI 1.53 for Mac). A one-way analysis of variance, using crop rotation as a fixed effect was undertaken with all of the data. All tests were acknowledged as significant at $P \leq 0.05$. The post-hoc Tukey's Honest Significant Difference (HSD) test was used to compare means.

## 3.4 Results

### 3.4.1 Plant biomass

Seed, pod and root biomass did not differ between chickpea, lentil and pea ($P > 0.05$) (Table 3.2). In chickpea the majority of plant biomass was allocated to leaves and seeds ($P=0.003$) (Table 3.2). Lentil and pea had the greatest percentages of biomass allocated to seed and stems. The root:shoot ratio of the three pulse crops did not differ ($P > 0.05$) (Table 3.3).

### 3.4.2 Quantity, recovery and distribution of residue C and $^{13}C$

The C:N ratio of the plant parts did not differ among pulse crop treatments, apart from the seeds ($P=0.01$) (Table 3.4). Residue C and $^{13}C$ were greater in the ABG (leaves, stem and pods) plant residue (65 to 70% and 78 to 85%, respectively), than in the BG residues (30 to 35% and 15 to 22%, respectively), in all of the treatments, respectively (Fig. 3.3). Likewise, root derived C (18 to 22%) was greater than root C (12 to 13%) in the treatments.

Tables 3.5 and 3.6 show that the majority of residue C and $^{13}C$ in chickpea was allocated to the leaves and seeds. The majority of residue C and $^{13}C$ in lentil and pea was allocated to stems and seeds. Similarities in the distribution patterns of total C and $^{13}C$ in the ABG and BG components suggest that the distribution of recovered residue $^{13}C$ is representative of the distribution of total residue C. Recovery values do not include $^{13}CO_2$ lost from leaves, roots, and

SMB respiration, as it was not possible to quantify these fractions.

### 3.4.3 Quantity, recovery and distribution of N and $^{15}$N in plant parts

There was less residue N allocated to ABG (stems, leaves, pods) residue (32 to 47%) than BG residue (53 to 68%) (Fig. 3.4). Contrastingly, the distribution of ABG-$^{15}$N residue (75 to 82%) exceeded the distribution of BG-$^{15}$N residues (18 to 25%), in all of the crop rotations. Nitrogen and $^{15}$N derived from rhizodeposition was greater than the root N and $^{15}$N in all of the rotations ($P$>0.05). The distribution patterns of the recovered residue $^{15}$N follows the same trends as residue N, suggesting that residue $^{15}$N is representative of total residue N.

The majority of plant N was allocated to seed and rhizodeposits in all three continuous pulse crop systems (Table 3.7). The majority of plant $^{15}$N was allocated to leaves and seed in chickpea (Table 3.8). Lentil $^{15}$N was dispersed primarily to seed, stems and leaves; pea allocated the majority of $^{15}$N to seed. Recovery of $^{15}$N was greater than 70% and did not differ among the treatments ($P$>0.05).

### 3.4.4 Soil pool biomass and C:N ratio

The mass of the HF, LF and VLF that was isolated from each of the soil cores varied significantly from one another ($P$<0.0002) (Table 3.9). The HF, LF and VLF compromised 84 to 89%, 1.3% to 2.2% and 0.003 to 0.004% of the soil in the cores, respectively. There was no difference between treatments for any of the soil fractions ($P$>0.05). A greater proportion of SOM was recovered in the HF than the LF, and the amount of SOM in the LF exceeded the amount recovered in the VLF (Table 3.9). The percent C and N did not vary between treatments in any of the soil pools or in the bulk soil ($P$>0.05). The VLF had the highest percent C and N followed by the LF and then the HF (Table 3.9).

The C:N ratio of the SOM pools and the SMB did not differ between chickpea, lentil and pea after three years of monocropping ($P$>0.05) (Table 3.10).

### 3.4.5. Quantity, recovery and distribution of C and $^{13}$C in different soil pools

The soil C and $^{13}$C did not vary between the treatments in any of the soil pools ($P$>0.05) (Tables 3.11 & 3.12). The amount of C and $^{13}$C per core in each soil pool are proportional to the size of the corresponding soil pool. The HF contributed the most C and $^{13}$C to the soil because of

the larger proportion of the HF in the soil, despite the fact that the %C of the VLF and LF exceeded that of the HF.

Total recovery of $^{13}C$ varied from 0.6 to 5.7% ($P$>0.05) (Table 3.12). The similar distribution patterns between plant and soil C and $^{13}C$ in the bulk soil and soil pools demonstrate that the distribution of recovered $^{13}C$ is representative of the distribution of toal recovered C. Recovery values do not include $^{13}CO_2$ lost from leaves, roots, and microbial respiration, as it was not possible to quantify these fractions. It is also assumed that 100% of the added $^{13}C$ is derived from phtosynthesis.

### 3.4.6. Quantity, recovery and distribution of soil pool N and $^{15}N$

The soil N and $^{15}N$ did not vary between the continuous chickpea, lentil and pea systems in any of the soil pools ($P$>0.05) (Tables 3.13 & 3.14). Lentil had more $^{15}N$ allocated to the $NH_4^+$ pool than chickpea (P=0.029). The HF held the greatest N and $^{15}N$ content and distribution, while the VLF held the lowest N and $^{15}N$ content and distribution. The amount of N and $^{15}N$ per core in each soil pool are proportional to the size of the corresponding soil pool. The HF contributed the most N and $^{15}N$ to the soil because of the superiority of the HF in the soil, despite the fact that the %N of the VLF and LF exceeded that of the HF (Table 3.9).

Total recovery of $^{15}N$ was lowest in the soil of the chickpea (13.0%) and greatest in the soil of the lentil system (14.2%) ($P$>0.05) (Table 3.14). The distribution of soil $^{15}N$ is representative of the distribution of soil N as both $^{15}N$ and N follow the same patterns of distributions between soil pools.

**Table 3.2. Dry biomass (g core$^{-1}$) of each plant part and the distribution of biomass (%) as a percentage of total plant biomass in continuous (three year) chickpea, lentil, and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| | Biomass (g core $^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | **Leaves** | **Seeds** | **Pods** | **Stems** | **Roots** | **Total** |
| **Chickpea** | 4.1 (0.57) a† | 4.7 (1.45) | 1.3 (0.38) | 2.4 (0.6) b | 2.0 (0.23) | 14.5 (2.61) |
| **Lentil** | 2.3 (0.37) b | 3.8 (1.07) | 2.0 (0.56) | 3.7 (0.43) b | 1.6 (0.32) | 13.4 (2.44) |
| **Pea** | 2.0 (0.15) b | 6.7 (0.98) | 1.8 (0.28) | 6.1 (0.51) a | 1.9 (0.39) | 18.4 (2.07) |
| ***P value*** | 0.012 | 0.276 | 0.466 | 0.002 | 0.631 | 0.336 |
| | **Distribution of Plant Part Biomass (%)** | | | | | |
| **Chickpea** | 29.4 (4.56) a | 30.8 (3.54) | 8.3 (0.95) b | 16.1 (2.16) b | 15.4 (3.15) | 100 |
| **Lentil** | 17.2 (0.53) b | 26.2 (5.55) | 15.1 (2.24) a | 29.4 (3.42) a | 12.3 (1.50) | 100 |
| **Pea** | 11.3 (0.99) b | 35.9 (2.18) | 9.7 (0.99) b | 33.1 (1.25) a | 9.9 (0.92) | 100 |
| ***P value*** | 0.003 | 0.278 | 0.027 | 0.002 | 0.233 | |

† Different letters following means (n=4) and (standard errors) indicate a significant difference between treatments within each plant part ($P$<0.05) according to Tukey's HSD test.

**Table 3.3. Root biomass-to-shoot biomass ratio of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| Crop Rotation | Root:Shoot |
|---|---|
| **Chickpea** | 0.19 (0.045) |
| **Lentil** | 0.14 (0.019) |
| **Pea** | 0.11 (0.011) |
| ***P value*** | 0.229 |

**Table 3.4. The C:N ratios of the plant parts of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| | Plant part C:N ratio | | | | |
|---|---|---|---|---|---|
| | **Leaves** | **Seeds** | **Pods** | **Stems** | **Roots** |
| **Chickpea** | 19.6 (2.35) | 13.2 (0.68) a† | 52.8 (6.25) | 46.8 (5.88) | 19.9 (0.52) |
| **Lentil** | 14.7 (1.82) | 10.8 (0.50) b | 36.5 (7.43) | 35.9 (4.44) | 19.8 (0.47) |
| **Pea** | 19.6 (1.18) | 10.8 (0.34) b | 54.4 (3.13) | 46.8 (2.94) | 21.8 (0.26) |
| ***P value*** | 0.160 | 0.011 | 0.184 | 0.146 | 0.283 |

† Different letters following means (n=4) and (standard errors) indicate a significant difference between treatments within each plant part ($P$<0.05) according to Tukey's HSD test.

### 3.4.7 Quantity and distribution of CdfR and NdfR

None of the C:N ratios of the rhizodeposits varied between chickpea, lentil and pea in any of the soil pools or bulk soil ($P$>0.05). However, the lentil C:N ratio tended to be lower than chickpea and pea except for in the SMB and VLF (Table 3.15).

The amount and distribution of CdfR and NdfR did not vary between any of the treatments in any of the soil pools or bulk soil ($P$>0.05) (Table 3.16 & 3.17). The greatest amount of CdfR was recovered in the HF soil. The LF CdfR exceeded the VLF CdfR ($P$>0.05). The greatest amount of NdfR was recovered in the HF for the chickpea and the $NO_3$-N for the lentil and pea ($P$>0.05) (Table 3.17). The amount of CdfR and NdfR per core in each soil pool is proportional to the size of the corresponding soil pool. The HF contributed the most CdfR and NdfR to the soil because of the dominance of the HF in the soil, and despite the fact that the %C and %N of the VLF and LF exceeded that of the HF (Table 3.9).

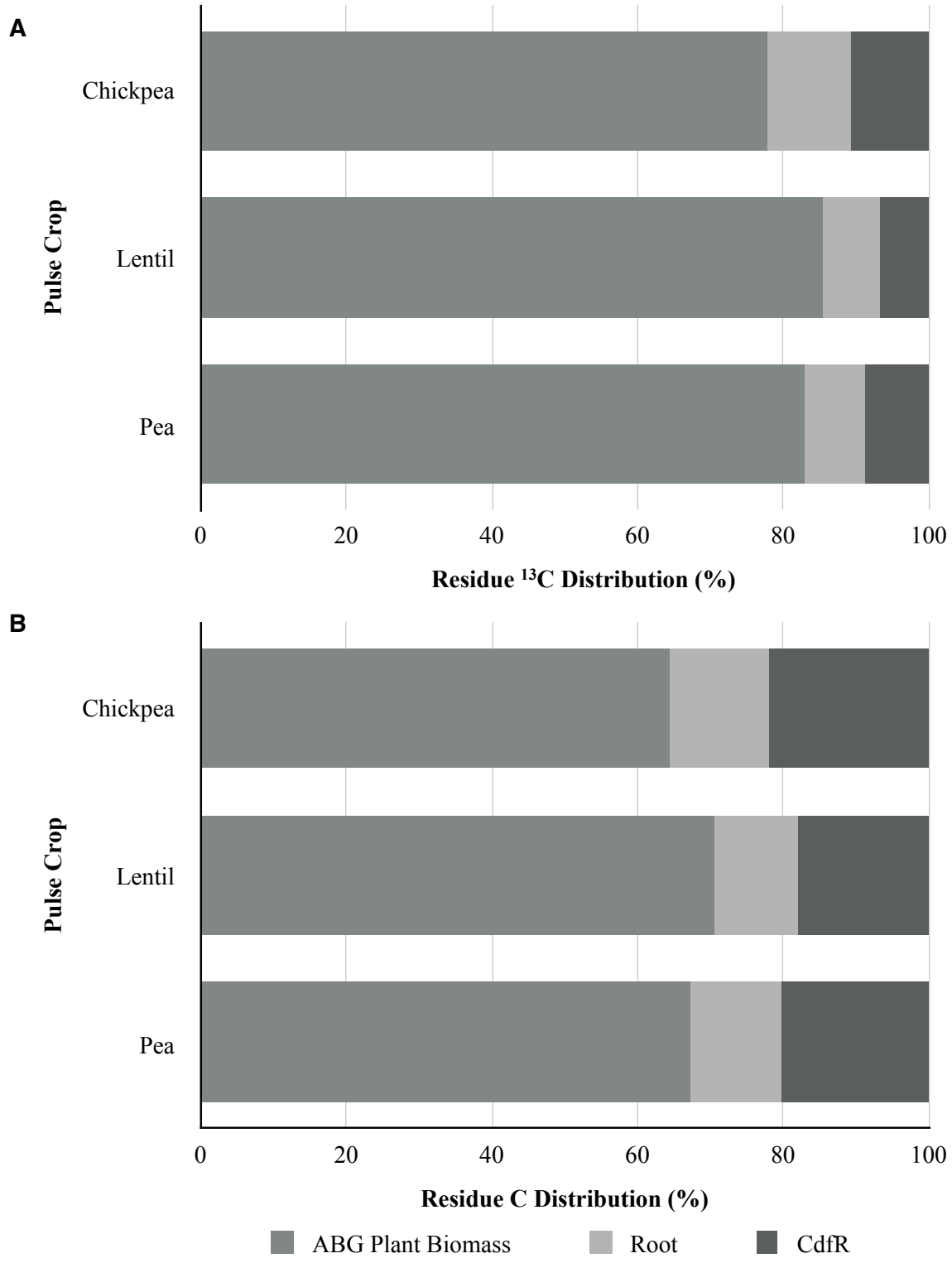


**Figure 3.3. Distribution of ABG residue $^{13}C$, root $^{13}C$ and $^{13}C$dfR (A) and total ABG residue C, root C and CdfR (B) as a proportion of total residue C of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}N$-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

**Table 3.5. Plant C (g core$^{-1}$) and the distribution of C (%) as a proportion of total plant C in plant parts and bulk rhizodeposition of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| | C (g core$^{-1}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Leaves** | **Seeds** | **Pods** | **Stems** | **ABG** | **Rhizodeposition‡,** | **Roots** | **BG Total** | **ABG+BG** |
| **Chickpea** | 1.5 (0.22) a† | 2.0 (0.61) | 0.5 (0.15) | 1.1 (0.25) b | 5.0 (1.12) | 1.1 (0.26) | 0.7 (0.067) | 1.8 (0.24) | 6.7 (1.20) |
| **Lentil** | 0.9 (0.15) ab | 1.4 (0.36) | 1.3 (0.24) | 1.4 (0.21) b | 4.6 (0.88) | 0.8 (0.22) | 0.6 (0.14) | 1.4 (0.16) | 5.9 (0.96) |
| **Pea** | 0.8 (0.064) b | 2.7 (0.41) | 0.7 (0.12) | 2.5 (0.22) a | 6.8 (0.71) | 1.0 (0.33) | 0.6 (0.14) | 1.6 (0.41) | 8.4 (1.0) |
| ***P value*** | 0.026 | 0.143 | 0.238 | 0.003 | 0.263 | 0.791 | 0.815 | 0.695 | 0.263 |
| | **Distribution of Total Plant C (%)** | | | | | | | | |
| **Chickpea** | 23.2 (4.06) | 27.8 (3.35) | 7.3 (0.73) | 14.6 (1.73) b | 73.5 (3.28) | 15.9 (2.43) | 11.3 (2.72) | 26.5 (3.28) | 100 |
| **Lentil** | 15.1 (1.29) | 19.5 (4.02) | 16.1 (2.25) | 24.1 (2.63) b | 75.7 (3.20) | 15.8 (5.31) | 9.4 (1.98) | 24.3 (3.20) | 100 |
| **Pea** | 9.7 (0.86) | 32.7 (2.92) | 8.7 (0.89) | 30.5 (1.33) a | 81.7 (3.63) | 11.3 (3.17) | 7.2 (0.68) | 18.3 (3.63) | 100 |
| ***P value*** | 0.101 | 0.805 | 0.171 | 0.038 | 0.263 | 0.595 | 0.163 | 0.263 | |

† Different letters following means and (standard errors) indicate a significant difference between treatments within each plant part ($P$<0.05) according to Tukey's HSD test.

‡ Quantity of CdfR determined by multiplying CdfR (%) calculated with the Janzen and Bruinsma equation (1989) and total C (mg core$^{-1}$)

**Table 3.6. Plant $^{13}C$ (mg core$^{-1}$) and the distribution of $^{13}C$ (%) as a proportion of total plant $^{13}C$ in plant parts and bulk rhizodeposition of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}N$-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| | $^{13}C$ (mg core$^{-1}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Leaves** | **Seeds** | **Pods** | **Stems** | **ABG Total** | **Rhizodep-osition‡** | **Roots** | **BG Total** | **Total** |
| **Chickpea** | 4.8 (0.78) | 5.6 (0.86) ab† | 1.4 (0.31) | 3.1 (0.72) b | 14.8 (2.05) b | 1.4 (0.61) | 1.3 (0.11) | 2.7 (0.70) | 17.6 (2.35) |
| **Lentil** | 3.4 (0.58) | 4.0 (1.17) b | 3.4 (0.81) | 5.3 (0.70) b | 16.0 (2.57) ab | 0.8 (0.42) | 1.2 (0.35) | 2.0 (0.42) | 18.0 (2.81) |
| **Pea** | 2.8 (0.27) | 10.0 (1.68 a | 2.8 (0.32) | 9.2 (1.34) a | 24.8 (3.11) a | 1.6 (0.42) | 1.6 (0.65) | 3.2 (1.22) | 28.0 (4.17) |
| ***P value*** | 0.092 | 0.022 | 0.065 | 0.005 | 0.047 | 0.673 | 0.843 | 0.653 | 0.081 |
| | **Recovery of $^{13}C$ (%)** | | | | | | | | |
| **Chickpea** | 1.6 (0.26) | 1.8 (0.28) ab | 0.4 (0.10) | 1.0 (0.24) b | 4.9 (0.68) b | 0.5 (0.20) | 0.4 (0.04) | 0.9 (0.18) | 5.8 (0.54) |
| **Lentil** | 1.1 (0.19) | 1.1 (0.39) b | 1.1 (0.27) | 1.7 (0.23) b | 5.3 (0.85) ab | 0.5 (0.26) | 0.4 (0.12) | 0.9 (0.21) | 6.2 (0.82) |
| **Pea** | 0.9 (0.09) | 3.3 (0.55) a | 0.9 (0.11) | 3.0 (0.44) a | 8.2 (1.02) a | 0.3 (0.14) | 0.5 (0.21) | 0.8 (0.17) | 9.0 (1.12) |
| ***P value*** | 0.092 | 0.022 | 0.065 | 0.005 | 0.047 | 0.673 | 0.843 | 0.653 | 0.081 |
| | **Distribution of Total Recovered Plant $^{13}C$ (%)** | | | | | | | | |
| **Chickpea** | 27.8 (3.78 a | 32.0 (2.83) | 7.5 (0.70) b | 17.2 (2.69) b | 84.6 (3.37) | 7.5 (3.16) | 7.9 (1.14) | 15.4 (3.37) | 100 |
| **Lentil** | 18.7 (0.61) ab | 20.3 (4.85) | 19.1 (2.04) a | 33.2 (6.16) b | 88.6 (1.69) | 5.2 (2.37) | 6.3 (1.20) | 11.4 (1.69) | 100 |
| **Pea** | 10.3 (0.90) b | 36.2 (4.48) | 10.7 (1.05) b | 35.0 (1.84) a | 89.7 (3.07) | 5.2 (2.59) | 5.1 (1.28) | 10.3 (3.07) | 100 |
| ***P value*** | 0.004 | 0.623 | 0.005 | 0.005 | 0.445 | 0.730 | 0.163 | 0.445 | |

† Different letters following means and (standard errors) indicates a significant difference between treatments within each plant part ($P$<0.05) according to Tukey's HSD test.

‡ Quantity of $^{13}$CdfR determined by multiplying CdfR (%) calculated with the Janzen and Bruinsma equation (1989) and total $^{13}C$ (mg core$^{-1}$)

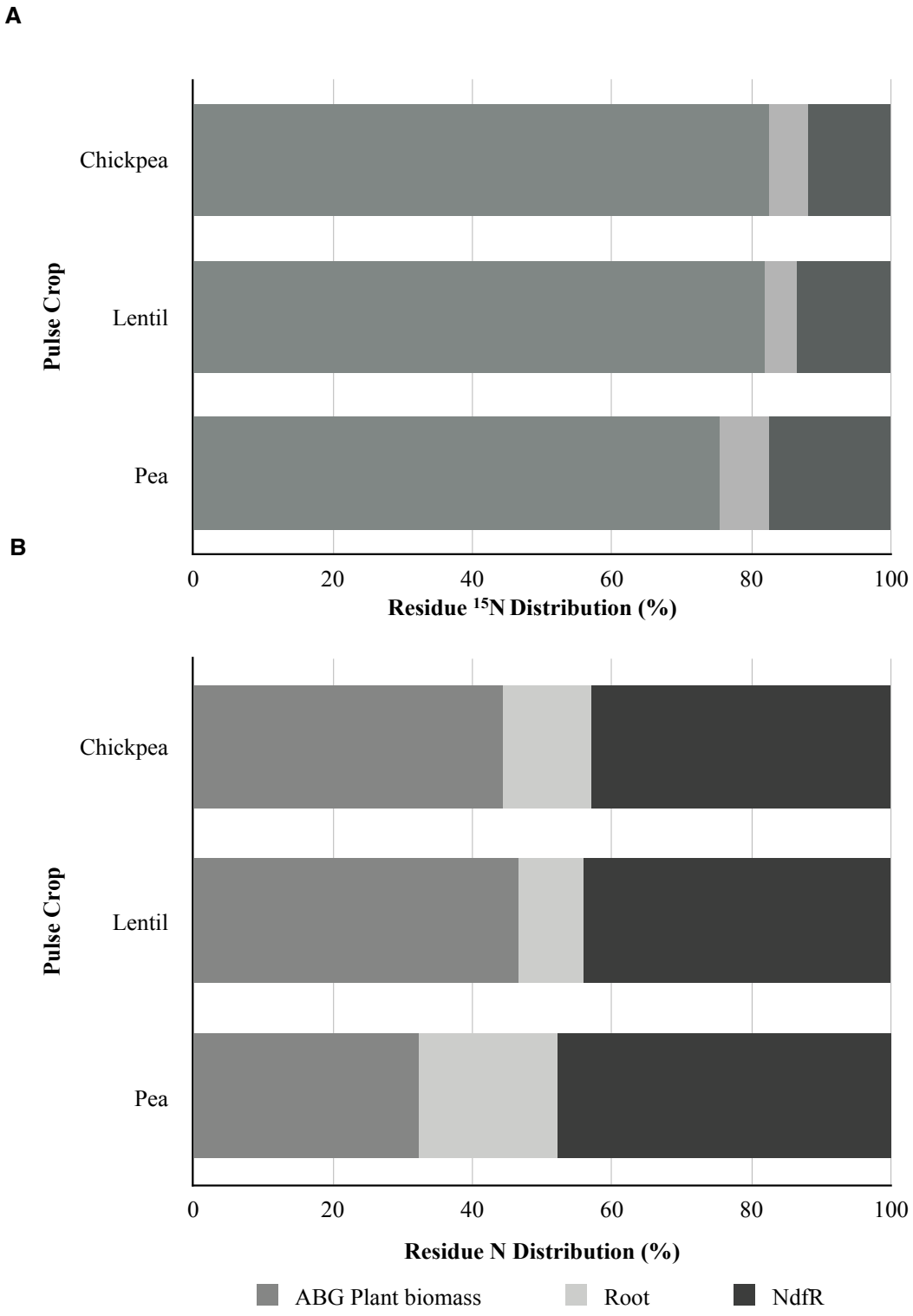


**Figure 3.4. Distribution of ABG residue $^{15}N$, root $^{15}N$ and $^{15}NdfR$ (A) and total ABG residue N, root N and NdfR (B) as a proportion of total residue N of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}N$-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

**Table 3.7. Plant N (mg core$^{-1}$) and the distribution of N (%) as a proportion of total plant N, in the plant parts and bulk rhizodeposition of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| | N (mg core$^{-1}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Leaves** | **Seeds** | **Pods** | **Stems** | **ABG Total** | **Rhizodeposition ‡** | **Roots** | **BG Total** | **ABG +BG Total** |
| **Chickpea** | 81.1 (14.83) | 146.4 (39.20) | 9.9 (2.39) | 23.4 (6.44) b† | 260.8 (54.01) | 116.8 (36.83) | 34.38 (3.43) | 149.1 (37.15) | 409.9 (76.12) |
| **Lentil** | 63.4 (13.16) | 144.6 (40.13) | 27.2 (9.86) | 45.1 (3.96 a | 280.3 (53.75) | 128.5 (43.37) | 25.79 (6.53) | 154.3 (37.68) | 434.6(19.48) |
| **Pea** | 41.8 (5.49) | 252.5 (32.15) | 15.7 (4.00) | 54.3 (4.30) a | 364.3 (35.93) | 115.5 (35.69) | 28.26 (4.98) | 143.7 (31.10) | 508.0 (16.41) |
| ***P value*** | 0.117 | 0.118 | 0.197 | 0.005 | 0.325 | 0.874 | 0.503 | 0.869 | 0.489 |
| | **Distribution as a proportion of Total Plant N (%)** | | | | | | | | |
| **Chickpea** | 21.2 (4.91) a | 34.8 (3.62) | 2.4 (0.36) | 5.6 (1.28) b | 64.0 (7.06) | 27.5 (7.31) | 8.5 (1.19) | 36.0 (7.06) | 100.00 |
| **Lentil** | 14.4 (2.43) ab | 32.6 (8.69) | 6.1 (1.93) | 10.4 (0.85) a | 63.4 (10.25) | 30.8 (11.40) | 5.8 (1.25) | 36.6 (10.25) | 100.00 |
| **Pea** | 8.1 (0.86) b | 49.8 (6.18) | 3.0 (0.72) | 10.6 (0.58) a | 71.6 (6.25) | 22.9 (7.15) | 5.5 (0.93) | 28.4 (6.25) | 100.00 |
| ***P value*** | 0.039 | 0.767 | 0.161 | 0.006 | 0.702 | 0.712 | 0.081 | 0.695 | - |

† Different letters following means and (standard errors) indicate a significant difference between treatments within each plant part ($P$<0.05) according to Tukey's HSD test.

‡ Quantity of NdfR determined by multiplying NdfR (%) calculated with the Janzen and Bruinsma equation (1989) and total N (mg core$^{-1}$)

**Table 3.8. Quantity of plant $^{15}N$ (mg core$^{-1}$), the recovery of $^{15}N$ (%) and the distribution of the recovered $^{15}N$ (%) as a proportion of total plant $^{15}N$, in the plant parts and bulk rhizodeposition of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}N$-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| | Leaves | Seeds | Pods | Stems | ABG Total | Rhizodepo-sition‡ | Roots | BG Total | ABG +BG Total |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | $^{15}N$ (mg core$^{-1}$) | | | | |
| **Chickpea** | 4.8 (0.48) | 6.8 (0.17) b† | 0.6 (0.06) | 2.2 (0.47) | 14.3 (1.16) | 1.0 (0.49) | 0.5 (0.086) | 1.5 (0.5) | 15.9 (1.02) |
| **Lentil** | 3.3 (0.42) | 4.4 (1.48) b | 0.8 (0.20) | 3.5 (0.81) | 12.1 (0.34) | 1.6 (1.07) | 0.3 (0.11) | 1.9 (1.0) | 14.0 (1.01) |
| **Pea** | 1.4 (0.22) | 11.0 (0.43) a | 0.6 (0.19) | 2.2 (0.21) | 15.3 (1.01) | 1.0 (0.36) | 0.4 (0.075) | 1.4 (0.3) | 16.6 (1.05) |
| ***P value*** | 0.086 | 0.002 | 0.476 | 0.206 | 0.087 | 0.803 | 0.515 | 0.816 | 0.238 |
| | | | | | Recovery of $^{15}N$ (%) | | | | |
| **Chickpea** | 24.7 (7.99) | 34.6 (0.88) b | 2.9 (0.33) | 11.1 (2.39) | 73.2 (5.92) | 5.3 (2.53) | 2.5 (0.44) | 7.8 (2.56) | 81.0 (3.66) |
| **Lentil** | 17.0 (2.13) | 22.6 (0.56) b | 4.3 (1.03) | 17.8 (4.11) | 61.7 (1.73) | 8.1 (5.44) | 1.8 (0.47) | 9.9 (5.06) | 71.6 (3.64) |
| **Pea** | 7.3 (1.11) | 56.3 (2.22) a | 3.1 (0.95) | 11.1 (1.06) | 77.9 (5.15) | 5.0 (1.83) | 1.9 (0.38) | 6.9 (1.54) | 84.8 (3.79) |
| ***P value*** | 0.086 | 0.002 | 0.475 | 0.207 | 0.803 | 0.803 | 0.503 | 0.803 | 0.238 |
| | | | | | Distribution of Total Recovered Plant $^{15}N$ (%) | | | | |
| **Chickpea** | 29.7 (7.96) a | 43.2 (2.90) ab | 3.6 (0.51) | 13.8 (2.92) | 90.3 (3.28) | 6.6 (3.19) | 3.1 (0.54) | 9.7 (3.28) | 100 |
| **Lentil** | 24.3 (3.98) ab | 32.4 (10.53) b | 6.0 (1.46) | 24.6 (4.72) | 87.3 (5.50) | 10.1 (6.15) | 2.6 (0.79) | 12.7 (5.50) | 100 |
| **Pea** | 7.9 (0.90) b | 65.3 (3.36) a | 2.8 (0.39) | 12.7 (0.77) | 91.8 (1.79) | 5.9 (2.16) | 2.2 (0.44) | 8.2 (1.79) | 100 |
| ***P value*** | 0.043 | 0.018 | 0.089 | 0.054 | 0.879 | 0.761 | 0.575 | 0.695 | - |

† Different letters following means and (standard errors) indicate a significant difference between treatments within each plant part ($P<0.05$) according to Tukey's HSD test.

‡ Quantity of CdfR determined by multiplying CdfR (%) calculated with the Janzen and Bruinsma equation (1989) and total C (mg core$^{-1}$) in the corresponding soil pool.

‡ Quantity of $^{15}$NdfR determined by multiplying NdfR (%) calculated with the Janzen and Bruinsma equation (1989) and total $^{15}N$ (mg core$^{-1}$)

**Table 3.9. The proportion (%) of the HF, LF and VLF SOM in the bulk soil, on a mass basis, and the %C and %N in the HF, LF and VLF SOM matter in the soil cores of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| | HF | | | LF | | | VLF | | |
|---|---|---|---|---|---|---|---|---|---|
| | %Mass | %C | %N | %Mass | %C | %N | %Mass | %C | %N |
| **Chickpea** | 86.8 (1.10 | 1.0 (0.29) | 0.1 (0.03) | 1.9 (0.27) | 2.5 (1.01) | 0.22 (0.08) | 0.003 (0.00070) | 25.7 (5.45) | 1.3 (0.32) |
| **Lentil** | 83.9 (2.82) | 1.0 (0.041) | 0.1 (0.003) | 2.2 (0.93) | 2.8 (0.27) | 0.25 (0.01) | 0.004 (0.00080) | 25.4 (5.60) | 1.4 (0.26) |
| **Pea** | 84.7 (1.70) | 1.0 (0.53) | 0.1 (0.06) | 1.3 (0.29) | 3.3 (1.65) | 0.29 (0.14) | 0.004 (0.00030) | 24.7 (12.34) | 1.3 (0.63) |
| ***P value*** | 0.186 | 0.669 | 0.888 | 0.623 | 0.351 | 0.301 | 0.340 | 0.987 | 0.920 |

**Table 3.10. The C:N ratios of the bulk soil, HF, LF and VLF organic matter and the SMB pool of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| | C:N Ratios | | | | |
|---|---|---|---|---|---|
| | **Bulk Soil** | **HF** | **LF** | **VLF** | **SMB** |
| **Chickpea** | 8.7 (0.14) | 8.5 (0.09) | 11.0 (0.23) | 19.4 (1.67) | 0.2 (0.11) |
| **Lentil** | 8.7 (0.093) | 8.5 (0.14) | 11.1 (0.55) | 17.9 (1.30) | 0.3 (0.011) |
| **Pea** | 8.8 (0.26) | 8.9 (0.38) | 11.5 (0.23) | 19.3 (0.84) | 0.3 (0.071) |
| ***P value*** | 0.899 | 0.418 | 0.756 | 0.685 | 0.861 |

**Table 3.11. Carbon (g core$^{-1}$) and C distribution (%), as a proportion of total soil C, in the bulk soil, the HF, LF and VLF organic matter, and in the SMB pool of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| | C (g core$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | **Bulk Soil** | **HF** | **LF** | **VLF** | **SMB** |
| **Chickpea** | 177.1 (4.01) | 128.6 (1.60) | 7.2 (0.73) | 0.1 (0.010) | 2.0 (0.73) |
| **Lentil** | 176.5 (7.87) | 119.8 (4.01) | 7.9 (2.77) | 0.1 (0.038) | 1.2 (0.37) |
| **Pea** | 183.4 (23.15) | 138.9 (17.45) | 7.3 (2.04) | 0.2 (0.020) | 0.9 (0.39) |
| ***P value*** | 0.931 | 0.459 | 0.966 | 0.356 | 0.423 |
| | **Distribution of total soil C (%)** | | | | |
| **Chickpea** | 100.0 (0.00) | 72.8 (36.39) | 4.1 (2.03) | 0.06 (0.03) | 0.02 (0.02) |
| **Lentil** | 100.0 (0.00) | 68.4 (34.18) | 4.3 (2.16) | 0.07 (0.04) | 0.01 (0.01) |
| **Pea** | 100.0 (0.00) | 77.6 (38.78) | 3.7 (1.84) | 0.09 (0.05) | 0.01 (0.01) |
| ***P value*** | | 0.515 | 0.904 | 0.396 | 0.449 |

**Table 3.12. Quantity of $^{13}C$ (mg core$^{-1}$) the recovery of $^{13}C$ (%) and the distribution of the recovered $^{13}C$ (%), as a proportion of total soil $^{13}C$, in the bulk soil, the HF, LF and VLF organic matter, the SMB and inorganic N pools of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}N$-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| | $^{13}C$ (mg core$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | **Bulk Soil** | **HF** | **LF** | **VLF** | **SMB** |
| **Chickpea** | 2.0 (0.54) | 1.4 (0.70) | 0.2 (0.03) | 0.003 (0.0015) | 0.1 (0.06) |
| **Lentil** | 1.5 (0.43) | 0.0 (0.47) | 0.2 (0.11) | 0.0001 (0.00052) | 0.07 (0.11) |
| **Pea** | 2.2 (0.77) | 2.0 (1.22) | 0.1 (0.03) | 0.0005 (0.0013) | 1.5 (1.24) |
| ***P value*** | 0.736 | 0.174 | 0.461 | 0.488 | 0.493 |
| | **Recovery of $^{13}C$ (%)** | | | | |
| **Chickpea** | 0.7 (0.18) | 0.5 (0.23) | 0.1 (0.0005) | 0.0009 (0.0005) | 0.04 (0.19) |
| **Lentil** | 0.5 (0.14) | 0.0 (0.16) | 0.1 (0.0002) | 0.0005 (0.0002) | 0.02 (0.26) |
| **Pea** | 0.7 (0.27) | 0.7 (0.38) | 0.04 (0.0004) | 0.0002 (0.0004) | 4.3 (3.12) |
| ***P value*** | 0.092 | 0.767 | 0.438 | 0.856 | 0.711 |
| | **Distribution as a proportion of total soil $^{13}C$ (%)** | | | | |
| **Chickpea** | 100.0 (0.00) | 47.3 (23.65) | 10.6 (5.3) | 0.1 (0.0068) | 0.6 (0.42) |
| **Lentil** | 100.0 (0.00) | 8.37 (4.18) | 19.4 (9.7) | 0.1 (0.071) | 0.7 (0.50) |
| **Pea** | 100.0 (0.00) | 51.8 (25.90) | 11.1 (5.5) | 0.2 (0.10) | 12.5 (8.85) |
| ***P value*** | | 0.252 | 0.653 | 0.928 | 0.683 |

**Table 3.13. Quantity of N (g core$^{-1}$), the recovery of N (%) and the distribution of the recovered N (%), as a proportion of total soil N, in the bulk soil, the HF, LF and VLF organic matter, the SMB and inorganic N pools of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| | N (g core$^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Bulk Soil** | **HF** | **LF** | **VLF** | **$NH_4^+$** | **$NO_3^-$** | **SMB** |
| **Chickpea** | 20.3 (0.38) | 15.2 (0.23) | 0.6 (0.067) | 0.005 (0.81) | 0.05 (0.0032) | 0.2 (0.069) | 0.2 (0.05) |
| **Lentil** | 20.3 (0.79) | 14.0 (0.23) | 0.7 (0.29) | 0.007 (1.93) | 0.06 (0.0063) | 0.1 (0.028) | 0.2 (0.11) |
| **Pea** | 20.7 (2.17) | 15.5 (1.53) | 0.6 (0.17) | 0.008 (0.94) | 0.05 (0.0024) | 0.2 (0.056) | 0.2 (0.08) |
| ***P value*** | 0.975 | 0.509 | 0.903 | 0.384 | 0.840 | 0.455 | 0.988 |
| | **Distribution as a proportion of total soil N (%)** | | | | | | |
| **Chickpea** | 100.0 (0.00) | 74.8 (37.41) | 3.2 (1.61) | 0.03 (0.01) | 0.3 (0.13) | 1.1 (0.57) | 0.02 (0.01) |
| **Lentil** | 100.0 (0.00) | 69.35 (34.68) | 3.5 (1.78) | 0.03 (0.02) | 0.3 (0.14) | 0.7 (0.35) | 0.02 (0.01) |
| **Pea** | 100.0 (0.00) | 75.49 (37.75) | 2.9 (1.43) | 0.04 (0.02) | 0.3 (0.13) | 0.7 (0.37) | 0.02 (0.01) |
| ***P value*** | | 0.364 | 0.853 | 0.338 | 0.142 | 0.917 | 0.807 |

**Table 3.14. Quantity of $^{15}N$ (µg core$^{-1}$), the recovery of $^{15}N$ (%) and the distribution of the recovered $^{15}N$ (%), as a proportion of total plant $^{15}N$, in the bulk soil, the HF, LF and VLF organic matter, the SMB and inorganic N pools of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}N$-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| | $^{15}N$ (µg core$^{-1}$) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Bulk Soil** | **HF** | **LF** | **VLF** | **$NH4^+$** | **$NO3^-$** | **SMB** |
| **Chickpea** | 1488.9 (419.87) | 520.3 (103.91) | 85.3 (14.06) | 0.5 (0.20) | 12.7 (4.68) | 351.4 (140.63) | 98.8 (14.97) |
| **Lentil** | 1739.4 (575.30) | 605.5 (283.08) | 108.4 (27.11) | 0.4 (0.15) | 22.0 (3.84) | 254.6 (40.61) | 61.6 (71.37) |
| **Pea** | 1505.4 (487.16) | 415.9 (91.01) | 71.6 (14.71) | 0.4 (0.25) | 12.1 (3.41) | 420.4 (230.62) | 145.0 (94.64) |
| ***P value*** | 0.925 | 0.761 | 0.445 | 0.856 | 0.604 | 0.627 | 0.708 |
| | **Recovery of $^{15}N$ (%)** | | | | | | |
| **Chickpea** | 7.6 (2.14) | 2.6 (0.53) | 0.4 (0.072) | 0.003 (0.001) | 0.06 (0.024) | 1.8 (0.72) | 0.5 (0.008) |
| **Lentil** | 8.8 (2.94) | 3.1 (1.44) | 0.5 (0.14) | 0.002 (0.0008) | 0.1 (0.020) | 1.4 (0.21) | 0.3 (0.34) |
| **Pea** | 7.7 (2.75) | 2.1 (0.62) | 0.4 (0.10) | 0.002 (0.0012) | 0.06 (0.020) | 2.1 (1.13) | 0.7 (0.45) |
| ***P value*** | 0.925 | 0.767 | 0.434 | 0.622 | 0.189 | 0.760 | 0.725 |
| | **Distribution of total soil $^{15}N$ (%)** | | | | | | |
| **Chickpea** | 100.0 (0.00) | 39.9 (19.93) | 7.0 (3.52) | 0.03 (0.016) | 0.9 (0.42) b† | 24.1 (12.05) | 0.2 (0.15) |
| **Lentil** | 100.0 (0.00) | 30.3 (15.16) | 9.7 (4.84) | 0.02 (0.011) | 1.8 (0.88) a | 17.3 (8.67) | 0.02 (0.02) |
| **Pea** | 100.0 (0.00) | 66.1 (33.03) | 8.3 (4.16) | 0.03 (0.014) | 1.5 (0.77) ab | 25.3 (12.68) | 18.7 (13.21) |
| ***P value*** | | 0.612 | 0.867 | 0.623 | 0.029 | 0.924 | 0.713 |

† Different letters following means and (standard errors) show a significant difference between treatments within each plant part ($P$<0.05) according to Tukey's HSD test.

**Table 3.15. The C:N ratios of rhizodeposits in the bulk soil, HF, LF and VLF organic matter and the SMB pool of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| | C:N Ratios | | | | |
|---|---|---|---|---|---|
| | **Bulk** | **HF** | **LF** | **VLF** | **SMB** |
| **Chickpea** | 12.3 (4.65) | 9.3 (0.43) | 15.5 (2.65) | 38.7 (16.82) | 15.5 (5.73) |
| **Lentil** | 7.6 (2.26) | 3.4 (0.38) | 13.0 (4.99) | 63.7 (35.78) | 59.6 (59.60) |
| **Pea** | 26.9 (22.19) | 39.9 (2.48) | 11.2 (1.34) | 10.0 (9.96) | 34.8 (17.05) |
| ***P value*** | 0.533 | 0.208 | 0.667 | 0.402 | 0.267 |

**Table 3.16. CdfR (mg core$^{-1}$) and distribution of CdfR (%), as a proportion of plant C, in the bulk soil, in the HF, LF and VLF organic matter, and in the SMB pool of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| | CdfR (mg core$^{-1}$) ‡ | | | | |
|---|---|---|---|---|---|
| | **Bulk Soil** | **HF** | **LF** | **VLF** | **SMB** |
| **Chickpea** | 1032.2 (231.64) | 659.4 (338.76) | 90.4 (9.28) | 1.4 (0.0035) | 3.2 (1.28) |
| **Lentil** | 784.2 (43.37) | 111.1 (111.06) | 126.9 (61.80) | 0.8 (0.0029) | 0.8 (0.78) |
| **Pea** | 973.7 (35.69) | 866.4 (587.63) | 63.2 (19.63) | 0.6 (0.0080) | 12.6 (11.28) |
| ***P value*** | 0.805 | 0.274 | 0.515 | 0.714 | 0.679 |
| | **Distribution of CdfR as a proportion of plant C (%)** | | | | |
| **Chickpea** | 18.1 (2.96) | 12.3 (6.65) | 1.8 (0.34) | 0.02 (0.0084) | 0.04 (0.01) |
| **Lentil** | 18.4 (6.45) | 2.0 (2.02) | 2.1 (0.83) | 0.02 (0.0033) | 0.003 (0.003) |
| **Pea** | 13.0 (5.14) | 11.7 (7.60) | 0.9 (0.29) | 0.01 (0.014) | 0.1 (0.05) |
| ***P value*** | 0.715 | 0.426 | 0.349 | 0.829 | 0.530 |

‡ Quantity of CdfR determined by multiplying CdfR (%) calculated with the Janzen and Bruinsma equation (1989) and total C (mg core$^{-1}$) in the corresponding soil pool.

**Table 3.17. NdfR (mg core$^{-1}$) and distribution of NdfR (%), as a proportion of plant N, in the bulk soil, the HF, LF and VLF organic matter, the SMB and inorganic N pools of continuous (three year) chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of four cores and (standard errors).**

| | NdfR (mg core$^{-1}$) ‡ | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Bulk** | **HF** | **LF** | **VLF** | **$NH4^+$** | **$NO3^-$** | **SMB** |
| **Chickpea** | 116.8 (36.83) | 66.2 (15.15) | 6.1 (0.45) | 0.04 (0.014) | 1.0 (0.55) | 56.7 (11.16) | 0.2 (0.0087) |
| **Lentil** | 128.5 (43.37) | 44.8 (21.07) | 7.9 (1.87) | 0.03 (0.012) | 1.3 (0.15) | 57.1 (9.57) | 0.01 (0.0065) |
| **Pea** | 115.5 (35.69) | 45.8 (17.00) | 5.6 (1.30) | 0.04 (0.024) | 0.8 (0.19) | 62.0 (14.01) | 0.3 (0.20) |
| ***P value*** | 0.967 | 0.647 | 0.480 | 0.831 | 0.591 | 0.951 | 0.662 |
| | **Distribution of NdfR as a proportion of plant N (%)** | | | | | | |
| **Chickpea** | 42.1 (15.62) | 23.3 (5.47) | 2.2 (0 .29) | 0.02 (0.0071) | 0.3 (0.06) | 21.4 (6.76) | 1.0 (0.33) |
| **Lentil** | 62.7 (37.37) | 23.7 (16.49) | 2.6 (0.41) | 0.01 (0.0092) | 0.5 (0.20) | 25.3 (12.09) | 1.1 (0.71) |
| **Pea** | 32.7 (10.70) | 12.7 (5.03) | 1.5 (0.38) | 0.01 (0.0079) | 0.2 (0.12) | 17.5 (5.92) | 1.1 (0.41) |
| ***P value*** | 0.760 | 0.731 | 0.344 | 0.859 | 0.919 | 0.824 | 0.657 |

‡ Quantity of NdfR determined by multiplying NdfR (%) calculated with the Janzen and Bruinsma equation (1989) and total N (mg core$^{-1}$) in the corresponding soil pool.

## 3.5 Discussion

### 3.5.1 Aboveground and belowground partitioning of C and N

*3.5.1.1 Carbon*

The ABG and BG partitioning of C did not differ between chickpea, lentil and pea. A previous study found 33.7% of plant C was from BG contributions in mature pea (Wichern et al., 2007a). As a proportion of total plant C, this study found that 18.3% of pea plant C was from BG (roots + rhizodeposition) sources, which is lower than Wichern et al. (2007a), where soil depth was also 30 cm. However when seed is excluded from the ABG residue budget (i.e stems, leaves and pods only), pea BG-C accounts for 32.7% of total residue C, which is comparable to the findings of Wichern et al. (2007a). The allocation of BG residue C in chickpea (35.5%) and lentil (29.5%) were also comparable to Wichern et al. (2007a). In the same study, CdfR compromised 29.6% of total pea plant C, more than double the amount in this study where pea CdfR was 11.3% of plant C (Wichern et al., 2007), but again when seed is excluded, CdfR accounts for 20.2% of residue C.

The turnover of more labile root derived residue by the SMB can result in reduced decomposition of more recalcitrant residues and SOM (Lekkerkerk et al., 1990; Torbert et al., 2000; Johnson et al., 2006). In chickpea and lentil, the proportion of SMB-CdfR is less than in pea where the SMB-C pool is smaller than in chickpea and lentil. This suggests that soil microbial communities consumed readily available CdfR in chickpea and lentil at a quicker rate, than in pea. This theory is supported by CdfR being able to supply 1.5 to 3 times more C to the SOC pool than shoot derived C (Johnson et al., 2006). In the continuous pea, SMB-CdfR made up 1.4% of the SMB-C which is less than the findings of Wichern et al. (2007a), where SMB-CdfR was 37% of SMB-C. The continuous pea rotations here may have decreased soil productivity and resulted in the lower SMB-CdfR levels than in Wichern et al. (2007b) where pea was not grown continuously (Nayyar et al., 2009).

The lack of CdfR in the VLF is likely a result of the dynamics of captured residue C proposed by Comeau et al. (2013), which states that fresh residue is first incorporated into the VLF. The residues are then solubilized by the SMB and some partially decomposed plant material enters the LF. Further decomposition allows the residues to be incorporated into the HF.

Since fresh residues are first incorporated into the VLF, it is possible that the freshly incorporated labile rhizodeposits are quickly being utilized or stored in the SMB, thus contributing to the greater SMB-CdfR pool (Comeau et al., 2013). Furthermore, the low C:N ratio of pulse crop residues results in a greater availability of the residues to the soil microbial communities; however, higher phenol and alkaloid concentrations exist in some legumes and can result in a reduced mineralization rate of the newly incorporated CdfR, causing greater storage in the SMB-C pool and less storage in the VLF (Franzluebbers & Hill, 2005).

*3.5.1.2 The Role of Residue C:N Ratio*

Gan et al. (2011) found that chickpea root C:N was greater than lentil root C:N, which was greater than pea. None of the root C:N ratios differed here ($P$>0.05); however, they ranged from 19.8 to 21.9, which is comparable to the pea C:N ratio (18:1) found by Gan et al. (2011). The average pulse crop straw residue C:N ratio for chickpea, lentil and pea was 33.1, 24.6 and 32.9, respectively. These C:N straw ratios are more similar to oil seed C:N straw ratios and are much higher than the C:N straw ratios (17:1) for chickpea, lentil and pea, reported by Gan et al. (2011). However, the ABG C:N ratios here are in the range for the ABG residue C:N ratios for pulse crops of 25:1 to 40:1 reported by Stevenson and van Kessel (1996).

The C:N ratio of the rhizodeposits in each soil fraction was higher than the C:N ratio of the soil in each fraction, which suggests that the residues did not directly affect the soil C:N ratio; however, mineralization and immobilization of N are affected by the C:N ratio of the residue inputs (Comeau, 2012).

Biederbecker et al. (1994) showed a greater concentration of N in lentil residues resulted in a narrower C:N ratio in the LF-OM of a wheat-lentil rotation than in various wheat, flax, and fallow rotations, in Swift Current. This study found 2.2 g LF-C $kg^{-1}$ soil under a 15-year wheat-lentil rotation. This is higher than the 0.5 g LF-C $kg^{-1}$ soil recovered under the continuously grown lentil in this study. The higher LF-C under the wheat-lentil rotation is likely a result of improved soil quality (SOC, LF-C, mineralizable C and N) associated with rotating lentil and wheat, relative to monocultures (Zentner et al., 2001).

The lesser C:N ratio in the HF than in the LF is consistent with other literature (Gregorich et al., 2006; Gregorich and Beare, 2007; Comeau, 2012). The %C of the HF, LF and VLF are also consistent with a study where lentil and pea were grown on a Brown and Dark Brown

Chernozem (Comeau et al., 2013). The greatest amount of CdfR was recovered in the HF, followed by a lower amount in the LF and the least amount in the VLF; this agrees with the findings from the second growing season of lentil in the study by Comeau et al. (2013). Based on the dynamic of captured residue C proposed by Comeau et al. (2013), the higher %C in the VLF suggests that the majority of the crop residues in the soil are freshly incorporated residues.

*3.5.1.3 Nitrogen*

The ABG and BG partitioning of N did not differ between chickpea, lentil and pea. However, a greater amount of residue N in chickpea, lentil and pea was derived from BG residues (roots and rhizodeposits) (53.3 to 67.8%) than ABG (pods,leaves and stems) residues (32.2 to 46.7%). This compliments a previous study with pea, where 60.6% of residue N was from BG contributions (Arcand et al., 2013b). On a per plant basis, the pea seed biomass of the non-fertilized pea from Arcand et al. (2013b) was double the biomass of the pea seed recorded here. Moreover, pea root biomass exceeded pea pod biomass, which contrasts the results from Arcand et al. (2013b). In all three crop rotations seed was the greatest contributor to ABG biomass, which contrasts Saha et al. (2015), who reported that chickpea stem and pod biomass exceeded seed biomass.

In greenhouse pot studies, using the stem wick method with non continuous pea rotations, Mayer et al. (2003) and Mahieu et al. (2007) reported 12% and 9.7% to 11.7% NdfR as a proportion of total plant N, respectively, while Wichern et al. (2007a) reported 36.6%. This study found values more comparable to Wichern et al. (2007a) with NdfR consisting of 27.5% of total pea plant N. Chickpea (30.8%) and lentil (22.9%) were also comparable to pea in Wichern et al. (2007). The similar distribution of NdfR to the Wichern et al. (2007) study is unexpected, as the soil in the aforementioned study had not had a leguminous crop planted in 6 years. A reduction in BNF from continuous pea rotation can be expected to lower NdfR levels (Knight, 2012). Accordingly, pea tended to have lower bulk NdfR than chickpea and lentil.

In a previous study that used the stem wick method to supply $^{15}N$ urea to pea plants, the root:shoot ratio was 0.05 at maturity, and rhizodeposition contributed 7.5% of total plant N to the bulk soil (Arcand et al., 2013a). The greater root:shoot ratio of 0.1 in mature pea in this study likely played a role in the greater contribution of rhizodeposition (22.9%) to total plant N than in Arcand et al. (2013a). Other studies have demonstrated that root biomass is an indicator of BG

contributions of C and N to SOM (Molina et al., 2001; Johnson et al., 2006; Sainju and Lenssen, 2011).

Nayyar et al. (2009) found plant N concentrations of 28.3 mg $g^{-1}$ from an 11-year continuous pea rotation on a Black Chernozem. When converted to mg N $g^{-1}$, a comparable plant N concentration of 21.4 mg $g^{-1}$ was found in the 3-year continuous pea rotation studied here. Gan et al. (2010) reported 14% of plant N in the roots of chickpea, lentil and pea. This study found lower values here, with 5.5 to 8.5% of total plant N being allocated to the roots of continuous chickpea lentil and pea, at maturity.

The %N of the HF, LF and VLF are consistent with a study where lentil and pea were grown on a Brown and Dark Brown Chernozem (Comeau et al., 2013). Sangster (2010) recovered 2.4 mg inorganic N $kg^{-1}$ soil in a continuous field pea rotation and St. Luce et al. (2013) recovered 8.7 mg inorganic N $kg^{-1}$ soil under canola planted in a Dark Brown chernozem soil in Lethbridge, SK, with field pea planted as the previous crop. When converted to mg N $kg^{-1}$ soil, this study found 19.2, 13.4 and 14.1 mg inorganic N $kg^{-1}$ soil, under continuous chickpea, lentil and pea, respectively. In the Wichern et al. (2007a) study, where pea was not a continuous rotation, there was 17.3 mg inorganic N $kg^{-1}$ soil, which is higher than the 14.1 mg inorganic N $kg^{-1}$ soil recovered here. A greater presence of phenols and alkaloids, found in some legumes such as chickpea and certain types of pea, can increase soil inorganic N (Franzluebbers and Hill, 2005; Mbagwu et al., 2011; Tarzi et al., 2012).

In unfertilized pea, Arcand et al. (2013b) reported that $NO_3$-N made up 13% of total NdfR. In this study the $NO_3$-NdfR pool under pea was 54% of total NdfR and 49% and 44% of total NdfR for chickpea and lentil, respectively. Due to the continuous pulse crops grown on the soil cores, and pulse crop residues being more efficiently converted to SOM by the soil microbial communities, pulse crop residue from the previous rotation may have been available for mineralization as soon as soil microbial populations became active, causing a greater proportion of $NO_3$-NdfR to be present in the soil (Knight, 2012).

In the continuous chickpea, 0.1% of the SMB-N was NdfR, in lentil 0.005% of SMB-N was NdfR and in pea 0.15% of the SMB-N was NdfR. These proportions of SMB-NdfR are less than the findings of Wichern et al. (2007b), where 18% of pea SMB-N was NdfR. In this case, pulse crop residue from the previous rotation may have been available for mineralization as soon as SMB populations became active, causing a lower proportion of SMB-NdfR to be present in

the soil (Knight, 2012). The low proportion of SMB-N derived from rhizodeposition in this study suggests that the rhizodeposits are a lower quality than in previous studies where SMB-N consists of a higher proportion of NdfR (Wichern et al., 2007b). The broad range of rhizodeposition values across various studies as well as the ones here, indicate that edaphic factors, plant specific variability, as well as the variability in the frequency and length of labeling events can cause a notable amount of variation in studies investigating rhizodeposition.

### 3.5.2 Aboveground and BG partitioning of $^{13}C$ and $^{15}N$

#### *3.5.2.1 $^{13}C$*

The ABG and BG partitioning of $^{13}C$ did not differ between chickpea, lentil and pea. A greater distribution of recovered $^{13}C$ in the ABG residue (78 to 85%) than in the BG residue (15 to 22%) agrees with the findings of Wichern et al. (2007a). Wichern et al. (2007a) found BG-$^{13}C$ made up 17.6% of total plant $^{13}C$ in pea, which is greater than the 10.3% BG-$^{13}C$ recovered in pea in this study.

A total of 9.0% of the $^{13}C$ applied to the pea plants was recovered in the ABG and BG plant parts. This is low in comparison to the 32.4% recovery of $^{13}C$ in the mature pea plants in Wichern et al. (2007a). It is possible that microbial respiration was greater in this study and caused a greater loss of $^{13}C$ respiration (i.e as $^{13}CO_2$) and contributed to the incomplete recovery of $^{13}C$ (Nguyen, 2003; Kuzyakov and Schneckenberger, 2004; Jones et al., 2009). Furthermore, assimilation of $CO_2$ is lower in legumes than it is in cereals (Kuzyakov and Domanski, 2000). Thus, a lower rate of $^{13}CO_2$ assimilation also may have contributed to lower $^{13}CO_2$ recovery.

The allocation of recovered ABG-$^{13}C$ in pea plants in the Wichern et al. (2007a) study was greatest in the stem and leaves, followed by the seed. Similarly the distribution of lentil $^{13}C$ in this study was greatest in the stems; however unlike pea in Wichern et al. (2007a), the allocation of $^{13}C$ to seed exceeded that of the leaves in all three pulse crops here. A slightly greater amount of $^{13}C$ was recovered in the roots of pea than in the rhizodeposits and this agrees with the finding of Wichern et al. (2007a). According to Sangster et al. (2010), repeat-pulse $^{13}C$ labeling does not produce a homogeneous distribution of the $^{13}C$ label. The preferential allocation of $^{13}C$ to the ABG biomass, over the roots, in this study is also consistent with the findings of other $^{13}CO_2$ labeling studies (Comeau et al., 2013; Matus et al., 2014).

The heterogeneous distribution of the $^{13}C$ label must be taken into consideration when assessing $^{13}C$ residue transfer to soil. The continually increasing $^{13}C$ enrichment caused by using multiple injections of HCl, to produce multiple $CO_2$ spikes will also have contributed to the heterogeneity of the $^{13}C$ label distribution. The multiple injection technique was chosen to ensure the plants were exposed to adequate levels of $^{13}CO_2$.

*3.5.2.1 $^{15}N$*

The ABG and BG partitioning of $^{15}N$ did not differ between chickpea, lentil and pea. There was a greater distribution of $^{15}N$ in the ABG residue (75 to 82%) than the BG residue (18 to 25%) for all three treatments. This finding is consistent with Arcand et al. (2013b), where the ABG pea residue $^{15}N$ (66%) exceeded the BG (roots and rhizodeposits) residue $^{15}N$ (34%). Recovery of $^{15}N$ in pea plant components and soil pools (89.5%) is also on par with the recovery of $^{15}N$ in pea at maturity (83.2%) by Arcand et al. (2013a). Partial recovery of $^{15}N$ may be a result of $^{15}N$ loss via denitrification and volatilization of $^{15}NH_3$ from plant leaves (Zebarth et al., 1991; Mayer et al. 2003). Residual $^{15}N$ held in the stem wick apparatus as well as experimental errors in measuring plant part $^{15}N$ to calculate the total $^{15}N$ balance may have also contributed to incomplete $^{15}N$ recovery (Mayer et al., 2003). Consistent with this study's findings, previous studies using shoot labeling techniques have reported preferential $^{15}N$ enrichment of the ABG plant parts over the roots, as well as the heterogeneous distribution of $^{15}N$ residue (Russell and Fillery, 1996; Wichern et al., 2007a, b; Arcand et al., 2013a, b). One of the limitations of using $^{15}N$ shoot labeling techniques for tracking ABG and BG residue N to the soil is the imbalanced distribution of $^{15}N$ to the ABG and BG plant parts. The reduced allocation of $^{15}N$ to the roots is likely an outcome of applying the $^{15}N$-urea directly to the plant stem (Wichern et al., 2010). The heterogeneous distribution of $^{15}N$ within the plant roots is likely to have caused errors in the NdfR estimation (Khan et al., 2002). A study investigating the optimization of $^{15}N$ shoot labeling procedures reported unnodulated chickpea roots with 56% more $^{15}N$ enrichment than nodulated chickpea roots. This finding suggests that the assumption that $^{15}N$ enrichment of all recovered roots is representative of the enrichment of NdfR may not be valid, particularly for chickpea (Khan et al., 2002). The same study also points out that applying the enrichment of recovered roots to a soil fraction requires the ratio of nodule:root material in the soil fraction to be the same as the ratio of nodules:roots of total recovered roots. This assumption is not always

met due to differences in nodulation patterns in different species and varying environmental conditions.

The assumption that $^{15}N$ enrichment of all recovered roots is representative of the enrichment of NdfR can not be fully met in this study due to the likelihood of incomplete recovery of fine root material, the heterogeneous distribution of $^{15}N$ within the roots caused by shoot labeling techniques, and due to not considering the nodule:root ratio.

### 3.6 Conclusion

This experiment used $^{15}N$ and $^{13}C$ stable isotope techniques to examine the partitioning of C and N to the plants and soil in continuous pulse crop systems. The ABG residue C (pods, leaves and stems) was greater than BG (roots and rhizodeposits) residue C in chickpea, lentil and pea. The BG-N was greater than ABG-N (pods, leaves and stems) in all 3 pulses and the majority of BG-N and BG-C was allocated to rhizodeposition. The amount and distribution of C, $^{13}C$, N and $^{15}N$ did not vary between chickpea, lentil and pea in the bulk soil, HF, LF, VLF, SMB or inorganic N pools. The amount and distribution of CdfR and NdfR did not vary significantly between treatments in any of the soil pools. The majority of the NdfR was allocated to the $NO_3$ pool and the HF. The high $NO_3$-NdfR is likely a result of pulse crop residues from the previous pulse crop being available for mineralization as soon as the soil microbial population in the current crop became active.

The lack of variability between continuous chickpea, lentil and pea suggests that ABG and BG partitioning of C and N in these systems is similar.

This study drew similarities and differences to other studies that quantified rhizodeposition in legumes. Edaphic factors, plant species, and variation in the rate and length of labeling events can cause discrepancy in studies investigating rhizodeposition. The inconsistencies associated with different crop rotation studies were evident. The partitioning of C and N in the continuous chickpea, lentil and pea systems agree and disagree with previous continuous and non-continuous pulse crop rotation studies. It is clear that further investigation into the effects of pulse crop rotation on C and N dynamics is required.

# 4. THE INFLUENCE OF LEGUME CROPPING SEQUENCES ON ABOVEGROUND AND BELOWGROUND CARBON AND NITROGEN INPUTS IN PULSE CROP ROTATIONS

## 4.1 Abstract

Incorporating pulse crops in rotation with cereals can provide economic and environmental benefits to producers; however, a better understanding of the effects of pulse crop rotation on the aboveground (ABG) and belowground (BG) partitioning of C and N is required. The aim of this study was to estimate the ABG and BG partitioning of C and N inputs to soil from pulse crop rotations. Chickpea (CP), lentil (L) and pea (P), in rotation with mustard (M) or wheat (W) were enriched with $^{15}N$ and $^{13}C$ stable isotopes, to determine the ABG and BG distribution of C and N residues in plant parts and soil pools. The partitioning of ABG and BG-C and $^{13}C$ did not differ between pulse crop rotations ($P>0.05$). In the chickpea rotations (CP-W-CP, P-W-CP and P-M-CP), pea rotations (P-W-P and P-M-P) and the L-W-L rotation, BG-C made up 50.3%, 43.4% and 25.1% of total residue C (including roots and rhizodeposits), respectively. Belowground N made up 76.4%, 69.7% and 61.8% of total residue N (including roots and rhizodeposits) in the chickpea rotations, pea rotations and the L-W-L rotation, respectively. A greater proportion of residue N was allocated to the roots of the P-M-CP and the CP-W-CP rotations than the pea and lentil rotations ($P<0.05$). A greater proportion of residue $^{15}N$ was allocated to the roots of the P-M-CP rotation than the L-W-L and P-W-P rotations ($P<0.05$). The amount and distribution of C and $^{13}C$ did not vary between pulse crop rotations in the bulk soil, HF, LF, VLF, SMB or inorganic N pools. The amount and distribution of CdfR and NdfR did not differ between pulse crop rotations in any of the soil pools apart from a greater distribution of CdfR to the LF in the CP-W-CP and P-W-P rotations. Overall, the pulse crop rotations examined partition residue C and N to ABG and BG residues in a similar manner.

## 4.2 Introduction

The potential environmental and economic benefits of rotating pulse crops with cereals and oilseeds include N transfer to succeeding crops (Gan et al., 2011; Arcand et al., 2013b), improved SOC levels (Lemke et al., 2007; Moncada and Schaeffer, 2010) and reduced pests and disease cycles (MacWilliam et al., 2012). It is clear however, that the effectiveness of pulse crops in rotations with cereals is reliant upon the extent of N held in the roots and NdfR

(Anglade et al., 2015). Since N cycling is highly dependent upon the availability of C in the soil and vice versa, the effectiveness of pulse crops in rotation with cereals is equally dependent upon the extent of C held in the roots and CdfR, as it is for N (Vitousek et al., 2002; Knickers et al., 2011; Fornara and Tilman, 2012). The proportion of plant N transferred to the soil via rhizodeposition has been measured in many studies and can range from 4 to 71% (Fustec et al., 2010; Wichern et al., 2008). Belowground C studies have shown rhizodeposition makes up half of the 30% to 40% of net fixed C that is transferred to the soil (Kuzyakov and Domanski, 2000; Kuzyakov and Schneckenberger, 2004; Jones et al., 2009). However, decomposition of the CdfR to $CO_2$ by microorganisms results in a smaller fraction of the rhizodeposition staying in the soil (Nguyen, 2003; Kuzyakov and Schneckenberger, 2004; Jones et al., 2009).

Benefits from pulse crops were reported in sub-humid India where alternating wheat and maize with green gram (*Vigna radiata*) and pea throughout five cropping seasons, over three years increased soil mineral N from 5.53 μg N $g^{-1}$ soil to 7.13 μg N $g^{-1}$ soil. Pea and green gram also increased soil organic C (SOC) from 5.00 g C $kg^{-1}$ soil to 5.40 g C $kg^{-1}$ soil (Pandiaraj et al., 2015). Furthermore, Li et al. (2012) found continuous peanut (*Arachis hypogaea*) cropping in China led to a significant decrease in soil pH and change in bacterial and fungal soil communities. These effects on the soil in the continuous cropping sequence slowed the cycling of C and N and led to increased autointoxicant production. In contrast, Fernandez et al. (2015) found that intercropping wheat, oat (*Avena sativa*), and radish (*Raphanus sativus*) with lentil did not provide consistent agronomic or economic benefits, relative to non-pulse crop rotations, in a field study in Minnesota.

In order to better understand global C and N cycling, there is a need for simultaneous quantification of CdfR and NdfR. More recently there has been an increase in the development of methods to measure C and N rhizodeposition (Kuzyakov and Domanski, 2000; Wichern et al., 2008; Fustec et al., 2010). Repeat pulse labeling has been successfully used for quantifying C rhizodeposition and has been suggested as a cost-effective approach to quantifying C rhizodeposition in plants (Meharg, 1994; Kuzyakov and Domanski, 2000; Sangster et al., 2010; An et al., 2015). As for N rhizodeposition studies, the stem wick technique has been reported as highly effective for labeling plants with $^{15}N$ (Hertenberger and Wanek, 2004; Wichern et al., 2008, Arcand et al, 2013a,b). However, preferential enrichment of ABG plant parts is common with shoot labeling techniques (Hertenberger and Wanek, 2004; Yasmin et al., 2006). Stable

isotope techniques are recognized as standard and effective approaches to improve our understanding of the dynamics of ABG and BG inputs of C and N from pulse crop rotations (Wichern et al., 2008; Fustec et al., 2010).

The objectives of this study were to (1) evaluate the ABG and BG partitioning of C and N inputs to soil using $^{15}N$ and $^{13}C$, (2) examine C and N residue inputs to the soil of chickpea, lentil and pea in rotation with mustard or wheat and (3) assess the ABG and BG distribution of C and N residues to plant parts and soil pools.

## 4.3 Materials and Methods

### 4.3.1 Experimental design and planting

In August 2011, 24 intact soil cores were extracted from an Orthic Brown Chernozem from the Agriculture and Agri-Food Canada Semiarid Prairie Agricultural Research Centre (SPARC) in Swift Current, Saskatchewan, Canada (50°15′N, 107°44′W) (Ayers et al., 1985). The soil cores were 20 cm in diameter and 30 cm in depth. Table 4.1 shows the crop rotations in the field from which the cores were collected. In 2012 the cores were cold stored and frozen over the winter. The cores were dry until moved into the greenhouse for seeding.

The cores were arranged in a randomized complete block design in the greenhouses at the University of Saskatchewan. Eight pea (cv. CDC Meadow) seeds or eight chickpea (cv. CDC Cory) seeds or eight lentil seeds (cv. CDC Imvincible CL) were planted per soil core on August 14, 2014 and culled to 4 plants per pot following germination.

All pea and lentil seeds were treated with a peat-based inoculant containing *Rhizobium leguminosarum* bv. *viciae* (Nodulator®XL, Becker Underwood, Saskatoon, SK) immediately prior to seeding. All chickpea seeds were also treated with a peat-based inoculant for chickpea containing *Rhizobium leguminosarum* bv. *Bradyrhizobium* (Nodulator®XL, Becker Underwood, Saskatoon, SK) prior to seeding. All seeds were inoculated at five times the recommended rate. The plants were watered every other day. Half of the cores (20) were used as background natural abundance (NA) controls and were kept in a separate greenhouse to avoid contamination of $^{13}C$ and $^{15}N$. Measurements from the NA soils and plants were used in calculations [Eq. 4.1 & 4.2].

**Table 4.1. Crop rotation history of the pulse crop soil cores used in experiment two. Chickpea, lentil and pea were grown in the university greenhouses in 2013.**

| Crop grown in SPARC field trial | | | Crop grown in greenhouse study |
|---|---|---|---|
| 2009 | 2010 | 2011* | Winter/Spring 2013 |
| Wheat | Pea | Mustard | Pea |
| Wheat | Pea | Mustard | Chickpea |
| Wheat | Pea | Wheat | Pea |
| Wheat | Pea | Wheat | Chickpea |
| Wheat | Chickpea | Wheat | Chickpea |
| Wheat | Lentil | Wheat | Lentil |

*Soil cores were taken at the end of this rotation phase.
Number of cores for enrichment = 4 treatments x 3 reps + 2 treatments x 4 reps = 20 cores.
Number of cores for natural abundance = 4 treatments x 3 reps + 2 treatments x 4 reps = 20 cores**. Total number of cores = 40**

### 4.3.2 $^{15}$N labeling

The stem wick method fed a $^{15}$N-enriched urea solution to the plants (Russell and Fillery, 1996; with modifications by Mayer et al., 2003 and Mahieu et al., 2009). The stem wick method consists of drilling a 0.5 mm hole through the plant stem approximately 5 cm above the soil. A sewing needle was used to pull a 10 cm cotton thread through the drilled hole. Silicone tubing (0.76 mm i.d. × 4 cm length) covered the thread to reduce evaporation of the $^{15}$N solution and plasticine was used to hold the tubing against the plant. The two ends of the thread were placed into 0.35 mL 0.4 % (w/v) $^{15}$N enriched urea solution (98.2 atom% $^{15}$N), held in a 2 mL vial with a secured cap. The Teflon coated silicone septum in the cap had a space that allowed the tubing and thread to pass through it into the vial, with minimal solution losses via evaporation.

When required, 0.35 mL of the $^{15}$N enriched urea solution was injected through the cap using a needle and syringe. Labeling took place for seven weeks. During the first three weeks, the solution was applied every other day. Solution uptake slowed as the plants matured and was applied every four to six days. After the last of $^{15}$N enriched urea solution was taken up by the plants, 0.40 mL of deionized water was injected into the vials to encourage total uptake of the $^{15}$N-urea solution.

Due to the development of callus tissues where the stem had been drilled, all plants were re-wicked at least once, with some plants being re-wicked two or three times throughout the labeling period. It was evident that a callus had formed when uptake slowed significantly and the

stem became very hard in the area of the hole. A total of 10.6 mg $^{15}$N-urea was supplied to each plant from 21 to 70 days after sowing.

### 4.3.3 $^{13}$C labeling

Following Sangster et al. (2010), all the plants were labeled with $^{13}$C at the same time, in polymethyl methacrylate chambers that accommodated four soil cores each. The cores were exposed to a $^{13}$C-enriched atmosphere once per week for 20 to 60 minutes, depending on the rate of photosynthesis during the growing period. There were eight weekly labeling events, which began and ended 21 and 70 days after sowing, respectively. GLAD Press'n Seal Freezer® wrap (The Clorox Company, Oakland, CA) was used to isolate the soil from the enriched atmosphere, during each labeling event and prevents dilution of $^{13}$C by soil respiration. The same $^{13}$C labeling procedure followed in chapter 3 was repeated for this experiment. Each chamber was exposed to a total of 404.5 mg $^{13}$C over the eight labeling events.

### 4.3.4 Plant and soil analysis

Labeling ceased at maturity. The plants were harvested and separated into leaves, stems, pods and seed, five days after the final labeling event. The soil cores were dismantled and divided into 0 to 10 cm, 10 to 20 cm and >20 cm sections. All large visible roots were removed by hand and washed. The fine roots were removed by hand from the top 10 cm of the soil and washed. Approximately 85% of total root biomass was in the top 10 cm of the soil cores. The bulk soil, plant parts and roots were dried for two days at 60°C and then coarsely ground using a coffee grinder before fine grinding in a ball mill. All plant, root and bulk soil subsamples were analyzed for %C, %N, and atom% $^{13}$C and atom%$^{15}$N, using a Costech Elemental Combustion System (Costech Analytical 191 Technologies, Inc.) paired with a Delta V Advantage Mass Spectrometer (Thermo Fisher 192 Scientific Inc.).

Following Carter and Gregorich (2008), soil $NO_3^-$-N and $NH_4^+$-N were extracted from each soil core using 2*M* KCl. The extracts were quantified colorimetrically with a SmartChemTM 200 (Westco Scientific Instruments, Brookfield, CT). The same procedure (Stark and Hart, 1996) described in chapter 3.3.4 for determining the enrichment of $^{15}$N in the $NO_3^-$ and $NH_4^+$ pools in the KCl extracts was followed for this experiment.

The heavy fraction (HF) and light fraction (LF) organic matter pools were isolated by

mixing 50 g soil subsamples and 100 mL sodium iodide (NaI, density of 1.7 g $mL^{-1}$) for one hour on a shaker and then left to stand at room temperature for 48 hours (Gregorich and Beare, 2007). The subsamples were filtered through a vacuum filter unit with a 0.4 μm polycarbonate filter. The LF was further fractionated into very light fraction (VLF) and LF, following the same procedure as for the HF but substituting deionized water for NaI, which has a density of 1.0 g $mL^{-1}$. The HF and LF subsamples were also encapsulated and analyzed for %C, %N, and atom%$^{13}$C and atom%$^{15}$N, with the Costech Elemental Combustion System (Costech Analytical 191 Technologies, Inc.) paired with a Delta V Advantage Mass Spectrometer (Thermo Fisher 192 Scientific Inc.).

The water extractable organic matter (WEOM) was isolated from the bulk soil by mixing subsamples of soil with 5 mM $CaCl_2$ for 10 minutes, at a 2:1 ratio (volume:dry mass equivalent) (Zolsnay, 1996). The extracts were filtered through a vacuum filter unit with a 0.4 μm polycarbonate filter.

The soil microbial biomass was isolated and the same chloroform fumigation method for soil microbial biomass extractions followed in chapter 3.3.4 was repeated for this experiment (Voroney et al., 2007). Enrichment of $^{15}$N and the concentration of N in the SMB extracts and the WEOM was determined following the Stark and Hart (1996) methodology for the recovery of $^{15}$N from persulfate digests. Enrichment of $^{13}$C and the concentration of C in the SMB extracts and the WEOM was determined by mass spectrometer analysis of the salt that remained in the vial after evaporation of the liquid extracts in a drying oven at 40°C.

### 4.3.5 Calculations

Nitrogen and C derived from rhizodeposition were calculated as (Janzen and Bruinsma, 1989):

$$\%NdfR = \frac{\text{atom\%15N Soil-atom\%15N background A}}{\text{atom\%15N Roots-atom\%15N background B}} \times 100 \qquad [4.1]$$

and

$$\%CdfR = \frac{\text{atom\%13C Soil-atom\%13C background A}}{\text{atom\%13C Roots-atom\%13C background B}} \times 100 \qquad [4.2]$$

where the atom% $^{13}C$ and $^{15}N$ values in the soil of plants grown under NA conditions is represented by background-A, and the atom% $^{13}C$ and $^{15}N$ values in the roots of plants grown under NA conditions is represented by background-B (Schmidtke, 2005). Separate %NdfR and %CdfR values were calculated for the bulk soil, the HF, LF, VLF, WEOM and the SMB pool. The %NdfR value was also calculated for the $NO_3$-N and $NH_4$-N soil pools. The amount of CdfR and NdfR (mg core$^{-1}$) was calculated by multiplying %CdfR and %NdfR in a specific soil pool, by total C and N (mg core$^{-1}$) in the respective soil pool. Rhizodeposition values are representative of net C and N rhizodeposition and do not consider reabsorption of C and N by the plant. It was assumed that there was a uniform distribution of $^{13}C$ and $^{15}N$ within the root system, that $^{13}C$ and $^{15}N$ enrichment was constant throughout growth, and that there was equal $^{13}C$ and $^{15}N$ enrichment of the recovered roots and rhizodeposits (Janzen and Bruinsma, 1989; Mayer et al., 2003b).

The distribution of plant C, N, $^{13}C$ and $^{15}N$ was calculated as a percentage, by dividing the amount of C, N, $^{13}C$ or $^{15}N$ (mg core$^{-1}$) in a specific plant part or the rhizodeposits by the sum amount of C, N, $^{13}C$ or $^{15}N$ (mg core$^{-1}$) in all of the plant parts and rhizodeposits, without inclusion of soil $^{13}C$ and $^{15}N$ (Russell and Fillery, 1996).

The distribution of C, N, $^{13}C$ and $^{15}N$ within the plant-soil system was calculated as a percentage by dividing the amount of C, N, $^{13}C$ or $^{15}N$ (mg core$^{-1}$) in a particular soil pool by the total amount of C, N, $^{13}C$ or $^{15}N$ (mg core$^{-1}$) in the bulk soil, as well as in all of the residue C, N, $^{13}C$ or $^{15}N$ (mg core$^{-1}$).

### 4.3.6 Statistical analysis

All statistical analyses were completed with the computer program R (version 2.15.2 GUI 1.53 for Mac). All data was subject to a one-way analysis of variance, using crop rotation as a fixed effect. All tests were acknowledged as significant at $P \leq 0.05$. Means were compared with the post-hoc Tukey's Honest Significant Difference (HSD) test.

## 4.4 Results

### 4.4.1 Plant biomass

Leaf, seed, pod, stem and total biomass did not differ between the chickpea, lentil or pea rotations (Table 4.2). Root biomass in the chickpea rotations exceeded that of the lentil and pea rotations ($P$=0.002). The majority of plant biomass was allocated to seed in all of the rotations except L-W-L where stem held the greatest biomass. The root:shoot ratio of the chickpea rotations exceeded those of the lentil and pea rotations ($P$>0.05) (Table 4.3).

### 4.4.2 Quantity, recovery and distribution of residue C and $^{13}C$

The C:N ratio of the ABG plant parts did not differ among pulse crop rotations ($P$>0.05) (Table 4.4). The root C:N ratio of CP-W-CP was lower than the root C:N ratio of all other rotations ($P$=0.009).

The majority of residue $^{13}C$ was allocated to the ABG plant parts (leaves, stems, pods) (97.0% to 99.8%) (Fig. 4.1a). In the chickpea rotations, a greater amount of BG residue (roots and rhizodeposition) $^{13}C$ was allocated to root-derived $^{13}C$ (0.4% to 2.7%) than root $^{13}C$ (0.3% to 0.7%); however in the pea and lentil rotations the opposite relationship was present.

A greater amount of residue C was allocated to the ABG residue (leaves, stems, pods) (52% to 75%) than BG residue (roots and rhizodeposition) (25% to 48%) in the CP-W-CP rotation as well as the pea and lentil rotations (Fig. 4.1b). The P-W-CP- and P-M-CP held a greater amount of residue C in the BG residue (52%). The majority of BG residue C was allocated to root derived C in all rotations. Less than 3.5% of recovered residue C was allocated to roots.

The majority of plant C was allocated to seed and rhizodeposition in all rotations except the lentil, where plant C was primarily found in the stems ($P$>0.05) (Table 4.5). The majority of plant $^{13}C$ was allocated to stems and seed (Table 4.6). A greater amount of recovered BG plant $^{13}C$ was in rhizodeposited $^{13}C$ than root $^{13}C$, in the chickpea rotations. The percent-recovered $^{13}C$ in the roots and total BG residue $^{13}C$ was greater in the CP-W-CP rotation than the lentil and pea rotations ($P$=0.006) (Table 4.6). There was greater recovery of total $^{13}C$ in the chickpea rotations and the L-W-L than the pea rotations ($P$=0.03). The similar patterns of distribution of C and $^{13}C$ in the ABG and BG plant and soil components indicate that the distribution of recovered plant

and residue $^{13}C$ is representative of the distribution of recovered residue C. The percent recovery does not include $^{13}CO_2$ lost from leaves, roots, and microbial respiration, as it was not possible to quantify these fractions. It is also assumed that 100% of the added $^{13}C$ is photosynthesized.

### 4.4.3 Quantity, recovery and distribution of plant part N and $^{15}N$

The majority of residue $^{15}N$ was allocated to the ABG plant parts (leaves, stems, pods) (70% to 97%) (Fig. 4.2a). There was a greater amount of residue $^{15}N$ allocated to the roots of the chickpea rotations and P-M-P rotation than the lentil and P-W-P rotation ($P$=0.006). Furthermore, the P-M-CP rotation had a greater amount of residue $^{15}N$ allocated to its roots than the other two chickpea rotations ($P$<0.001). A greater amount of BG residue (roots and rhizodeposition) $^{15}N$ was allocated to root-derived $^{15}N$ (2% to 25%) than root $^{15}N$ (0.4% to 5%).

The distribution of ABG (stems, leaves, pods) residue N (18% to 38%) was less than the BG-N (62% to 82%) in all pulse crop rotations (Fig. 4.2b).

Table 4.7 shows NdfR was greater than root N in all of the rotations and there was a greater amount of plant N allocated to the roots of the P-M-CP and CP-W-CP rotations, than the pea and lentil rotations ($P$<0.001).

The majority of plant $^{15}N$ was allocated to seed (Table 4.8). The amount of root $^{15}N$ in the CP-W-CP rotation exceeded that of the lentil and pea rotations ($P$=0.002). A greater percentage of added $^{15}N$ was recovered in the roots of the CP-W-CP rotation than in the lentil and pea rotations. The amount of BG plant $^{15}N$, as well as the percent-recovered $^{15}N$ was greater in the CP-W-CP rotation than the L-W-L rotation ($P$=0.04) (Table 4.8). The residue $^{15}N$ is representative of residue N, as the distribution of the recovered residue $^{15}N$ follows the same patterns as residue N.

### 4.4.4 Biomass and C:N ratio of soil pools

The C:N ratio of the VLF organic matter in all of the rotations exceeded the C:N ratio of all other soil pools (Table 4.9). The C:N ratio of the bulk CP-W-CP was greater than the C:N ratio of the L-W-L, P-M-P and P-W-CP rotations. The soil C:N ratio did not differ between the rotations in any of the SOM pools ($P$>0.05).

The HF, LF, VLF and WEOM compromised 57% to 86%, 2% to 5%, 0.04% to 0.1% and

0.04% to 0.1% of the soil in the cores, respectively. There was no difference in the percent mass of the soil fractions between crop rotations (Tables 4.10 & 4.11). The percent C and N did not differ between crop rotations in any of the soil pools or the bulk soil. The VLF had the highest percent C. Percent N was highest in the VLF, apart from the pea rotations. The %N in the WEOM of the pea rotations exceeded that of the VLF (Tables 4.10 & 4.11).

### 4.4.5. Quantity, recovery and distribution of soil pool C and $^{13}C$

The greatest amount of soil C and $^{13}C$ was recovered in the HF, followed by the LF, VLF and WEOM (Tables 4.12 & 4.13). Total recovery of applied $^{13}C$ in the HF, LF, VLF and WEOM ranged from 0.4% to 2.3% ($P$>0.05) (Table 4.13). The similar patterns of distribution of plant and soil C and $^{13}C$ in the bulk soil and soil pools indicate that the distribution of recovered $^{13}C$ is representative of the distribution of recovered C. The percent recovery values do not include $^{13}CO_2$ lost from leaves, roots, and microbial respiration, as it was not possible to quantify these fractions.

### 4.4.6. Quantity, recovery and distribution of soil pool N and $^{15}N$

The majority of the plant and soil N and $^{15}N$ was recovered in the HF (Table 4.14 & 4.15). There was more WEOM-N under the P-M-P and L-W-L rotations than the CP-W-CP rotation ($P$=0.02) (Table 4.14). The CP-W-CP had more $^{15}N$ in the $NH_4^+$-N pool than the lentil and pea rotations ($P$=0.01).

The recovered plant and soil $^{15}N$ is representative of soil N, as the distribution of the recovered soil $^{15}N$ followed the same pattern as soil N (Tables 4.14 & 4.15). Recovery of $^{15}N$ in the WEOM was greater in the P-M-P than the chickpea and lentil rotations ($P$=0.02) (Table 4.14). Recovery of $^{15}N$ in the $NH_4^+$-N pool was greater in the CP-W-CP than the lentil and pea rotations ($P$=0.01)

### 4.4.7 Quantity and distribution of CdfR and NdfR

None of the C:N ratios of the rhizodeposits differed between pulse crop rotations in any of the soil pools or bulk soil ($P$>0.05) (Table 4.16). The lentil rhizodeposit C:N ratio was lower than the chickpea and pea rotation rhizodeposit C:N ratios, except for in the VLF and WEOM ($P$>0.05).

The HF held the majority of soil pool CdfR. The lowest amount of CdfR was recovered in the WEOM (Table 4.17). There was a greater amount of CdfR allocated to the LF in the CP-W-CP and P-W-P rotations than in the L-W-L and P-M-P rotations ($P$=0.002). The majority of the NdfR was recovered in the HF (Table 4.18). The NdfR did not differ between pulse crop rotations in any of the soil pools or bulk soil ($P$>0.05).

**Table 4.2. Dry biomass (g core$^{-1}$) of each plant part of chickpea, lentil, and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | Biomass (g core $^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | **Leaves** | **Seeds** | **Pods** | **Stems** | **Roots** | **Total** |
| **P-W-CP ‡** | 6.6 (2.55) | 13.4 (3.79) | 4.9 (1.58) | 8.9 (1.77) | 2.0 (0.77) a† | 35.8 (8.81) |
| **P-W-P ‡** | 5.6 (1.84) | 14.3 (0.73) | 3.1 (0.18) | 10.6 (3.75) | 0.4 (0.16) b | 34.0 (3.07) |
| **P-M-CP ‡** | 7.9 (1.73) | 14.8 (5.21) | 5.6 (1.81) | 8.9 (1.1) | 2.4 (0.40) a | 39.6 (9.45) |
| **P-M-P ‡** | 5.5 (0.42) | 14.8 (0.74) | 4.9 (0.40) | 9.2 (1.42) | 0.5 (0.04) b | 34.8 (2.06) |
| **L-W-L §** | 10.3 (1.94) | 8.9 (0.27) | 7.4 (1.69) | 12.9 (3.35) | 0.6 (0.18) b | 48.9 (8.18) |
| **CP-W-CP §** | 10.3 (1.12) | 18.9 (3.59) | 12.4 (4.30) | 10.1 (0.45) | 3.9 (0.87) a | 55.6 (1.96) |
| ***P value*** | 0.197 | 0.282 | 0.163 | 0.792 | 0.002 | 0.134 |
| | **Distribution of Plant Part Biomass (%)** | | | | | |
| **P-W-CP** | 17.4 (2.51) | 36.8 (2.85) | 13.3 (1.74) | 25.5 (1.15) | 7.0 (3.04) a | 100 |
| **P-W-P** | 17.2 (6.64) | 42.5 (3.55) | 9.2 (0.36) | 29.9 (8.08) | 1.3 (0.53) b | 100 |
| **P-M-CP** | 20.2 (0.69) | 34.5 (7.10) | 13.3 (1.62) | 24.8 (5.37) | 7.3 (2.66) a | 100 |
| **P-M-P** | 16.0 (2.18) | 42.5 (0.97) | 13.9 (0.67) | 26.0 (2.58) | 1.5 (0.03) b | 100 |
| **L-W-L** | 25.2 (2.15) | 24.1 (4.45) | 17.7 (3.00) | 31.0 (3.86) | 1.9 (0.85) b | 100 |
| **CP-W-CP** | 18.3 (1.36) | 34.0 (6.40) | 22.5 (7.98) | 18.1 (0.36) | 7.1 (1.49) a | 100 |
| ***P value*** | 0.799 | 0.761 | 0.381 | 0.526 | 0.038 | |

† Different letters following means and (standard errors) show a significant difference between treatments within each plant part ($P$<0.05) according to Tukey's HSD test.

‡ n=3

§ n=4

**Table 4.3. Root biomass-to-shoot biomass ratio of chickpea, lentil, and pea grown in a greenhouse and labeled with $^{15}N$-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| Crop Rotation | Root:Shoot |
|---|---|
| **P-W-CP ‡** | 0.1 **(**0.034) a† |
| **P-W-P ‡** | 0.01 (0054) b |
| **P-M-CP ‡** | 0.1 (0.032) a |
| **P-M-P ‡** | 0.02 (0.00030 b |
| **L-W-L §** | 0.02 (0.0095 b |
| **CP-W-CP §** | 0.1 (0.017) a |
| ***P value*** | 0.045 |

† Different letters following means and (standard errors) indicate a significant difference between treatments within each plant part ($P$<0.05) according to Tukey's HSD test.
‡ n=3
§ n=4

**Table 4.4. The C:N ratios of the plant parts in each soil core where chickpea, lentil or pea were grown in a greenhouse and labeled with $^{15}N$-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | Plant part C:N ratio | | | | |
|---|---|---|---|---|---|
| | **Leaves** | **Seeds** | **Pods** | **Stems** | **Roots** |
| **P-W-CP ‡** | 12.2 (1.41) | 10.1 (1.06) | 56.2 (20.74) | 43.9 (2.64) | 30.4 (19.06) ab |
| **P-W-P ‡** | 16.6 (3.38) | 9.7 (0.90) | 59.3 (16.26) | 36.2 (10.35) | 89.5 (35.02) a |
| **P-M-CP ‡** | 16.6 (0.26) | 12.7 (0.24) | 72.0 (7.77) | 38.7 (1.89) | 12.7 (3.86) ab |
| **P-M-P ‡** | 12.3 (2.56) | 11.7 (5.23) | 35.7 (16.75) | 39.5 (23.02) | 54.9 (4.98) a |
| **L-W-L** § | 17.3 (0.46) | 9.8 (0.05) | 50.7 (1.10) | 46.2 (0.24) | 57.2 (27.85) ab |
| **CP-W-CP** § | 15.5 (1.64) | 13.0 (0.57) | 77.2 (9.73) | 42.3 (5.78) | 9.4 (1.71) b |
| ***P value*** | 0.983 | 0.314 | 0.414 | 0.882 | 0.009 |

‡ n=3
§ n=4

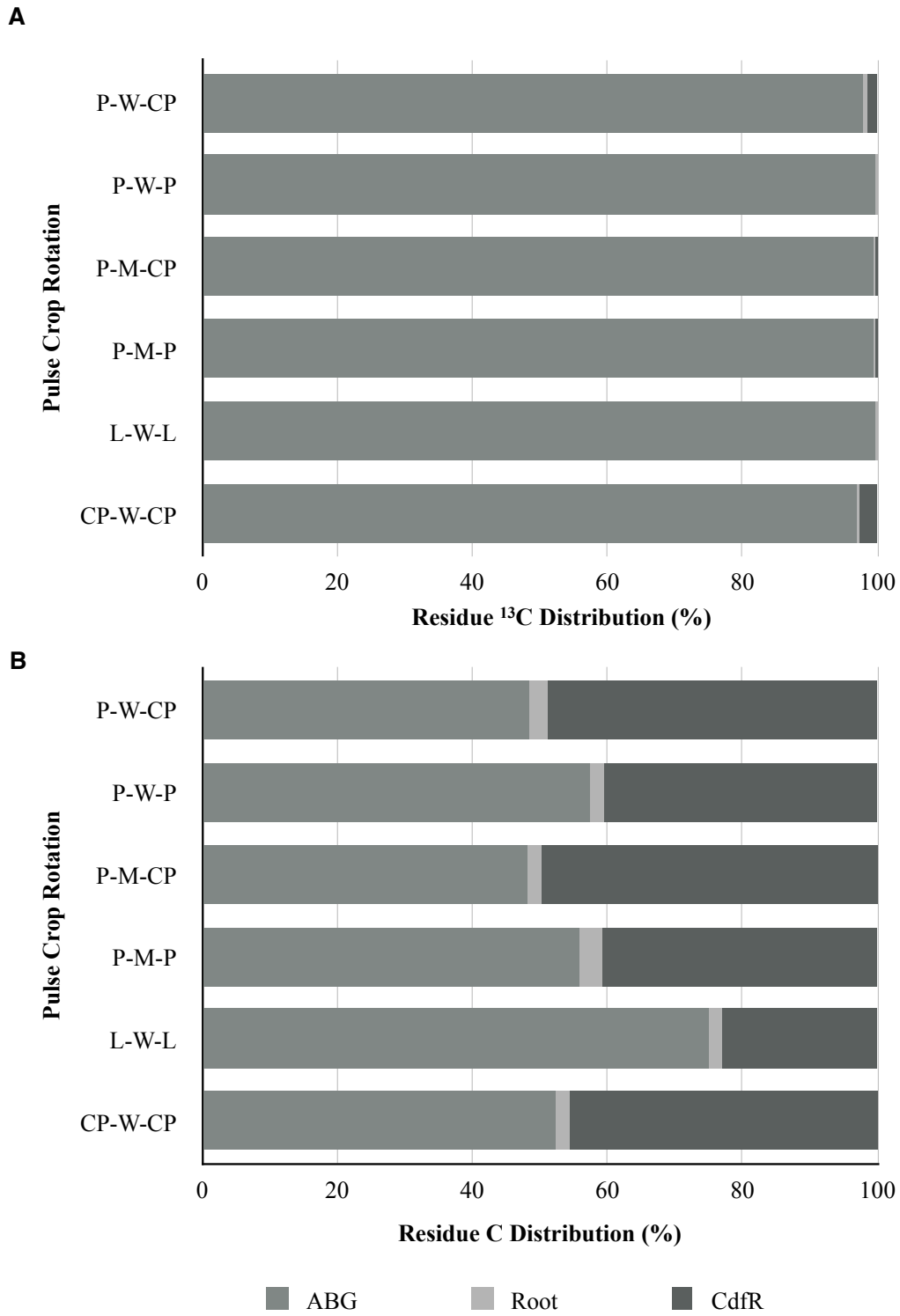


**Figure 4.1. Distribution of ABG residue $^{13}C$, root $^{13}C$ and $^{13}CdfR$ (A) and total ABG residue C, root C and CdfR (B) as a proportion of total residue C, of pulse crop rotations. Chickpea, lentil and pea were grown in a greenhouse and labeled with $^{15}N$-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

**Table 4.5. Plant C (g core$^{-1}$) and the distribution of C (%) as a proportion of total plant C in plant parts and bulk rhizodeposition, in chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | C (g core$^{-1}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Leaves** | **Seeds** | **Pods** | **Stems** | **ABG Total** | **Rhizodepo-sition¶** | **Roots** | **BG Total** | **ABG + BG Total** |
| **P-W-CP ‡** | 2.4 (0.0009) | 5.7 (0.002) | 2.1 (0.0007) | 4.0 (0.0007) | 14.1 (3.73) | 10.9 (5.00) | 0.5 (0.093) | 11.4 (5.04) | 25.5 (2.13) |
| **P-W-P ‡** | 2.2 (0.0007) | 5.7 (0.0002) | 1.3 (0.00008) | 4.5 (0.0002) | 13.7 (1.35) | 5.7 (1.25) | 0.3 (0.038) | 6.0 (1.22) | 19.7 (1.78) |
| **P-M-CP ‡** | 2.7 (0.0006) | 6.2 (0.002) | 2.3 (0.0008) | 3.9 (0.0007) | 15.2 (3.95) | 9.1 (1.47) | 0.4 (0.037) | 9.4 (1.44) | 24.6 (5.28) |
| **P-M-P ‡** | 1.5 (0.00004) | 4.4 (0.0002) | 1.5 (0.0002) | 3.7 (0.0006) | 11.1 (0.85) | 6.0 (1.83) | 0.5 (0.13) | 6.5 (1.77) | 20.0 (1.06) |
| **L-W-L §** | 3.9 (0.0008) | 3.7 (0.0001) | 3.1 (0.0007) | 5.9 (0.001) | 20.2 (3.42) | 4.0 (1.83) | 0.4 (0.24) | 4.4 (1.84) | 24.6 (4.02) |
| **CP-W-CP** | 3.9 (0.0004) | 8.3 (0.002) | 5.2 (0.002) | 4.2 (0.0002) | 21.6 (0.85) | 30.4 (23.76) | 0.6 (0.088) | 30.9 (23.79) | 52.5 (23.03) |
| ***P value*** | 0.222 | 0.750 | 0.155 | 0.633 | 0.166 | 0.603 | 0.817 | 0.598 | 0.362 |
| | **Distribution of Total Plant C (%)** | | | | | | | | |
| **P-W-CP** | 10.1 (4.44) | 22.8 (7.44) | 8.4 (3.15) | 15.9 (3.40) | 57.2 (18.25) | 40.9 (18.28) | 1.9 (0.21) | 42.8 (18.25) | 100 |
| **P-W-P** | 12.1 (5.24) | 29.6 (1.83) | 6.6 (0.54) | 21.7 (5.67) | 69.9 (4.85) | 28.5 (5.10) | 1.5 (0.29) | 30.1 (4.85) | 100 |
| **P-M-CP** | 11.0 (0.47) | 23.4 (5.59) | 8.9 (1.44) | 16.8 (2.56) | 60.0 (4.62) | 38.3 (4.26) | 1.7 (0.47) | 40.0 (4.62) | 100 |
| **P-M-P** | 10.3 (0.58) | 29.4 (2.29) | 10.0 (1.26) | 18.7 (3.32) | 68.4 (6.99) | 29.2 (7.39) | 2.4 (0.65) | 31.6 (6.99) | 100 |
| **L-W-L** | 18.3 (2.78) | 19.2 (3.69) | 12.1 (1.42) | 24.8 (4.96) | 79.7 (5.43) | 16.0 (5.55) | 1.6 (0.84) | 17.6 (5.43) | 100 |
| **CP-W-CP** | 11.3 (3.02) | 21.3 (6.15) | 16.8 (8.59) | 11.8 (2.86) | 61.2 (15.53) | 37.3 (15.85) | 1.5 (0.42) | 38.8 (15.53) | 100 |
| ***P value*** | 0.433 | 0.751 | 0.656 | 0.431 | 0.603 | 0.622 | 0.888 | 0.603 | |

‡ n=3

§ n=4

¶ Quantity of CdfR determined by multiplying CdfR (%) calculated with the Janzen and Bruinsma equation (1989) and total C (mg core$^{-1}$).

**Table 4.6. Plant $^{13}C$ (g core$^{-1}$) and the distribution of $^{13}C$ (%) as a proportion of total plant $^{13}C$ in plant parts and bulk rhizodeposition, in chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}N$-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | $^{13}C$ (g core$^{-1}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Leaves** | **Seeds** | **Pods** | **Stems** | **ABG Total** | **Rhizodeposi-tion¶** | **Roots** | **BG Total** | **ABG + BG** |
| **P-W-CP** ‡ | 31.3 (13.14) | 70.2 (20.55) | 25.9 (8.61) | 50.3 (9.75) | 177.7 (50.97) | 1.1 (0.54) | 0.6 (0.13) | 1.7 (0.65) | 179.4 (50.36) |
| **P-W-P** ‡ | 27.3 (8.79) | 72.1 (2.21) | 16.3 (0.84) | 56.1 (20.04) | 171.8 (18.78) | 0.1 (0.02) | 0.1 (0.06) | 0.2 (0.05) | 172.0 (18.75) |
| **P-M-CP** ‡ | 33.6 (7.57) | 72.7 (25.47) | 28.8 (9.89) | 46.2 (8.21) | 181.2 (48.42) | 0.4 (0.15) | 0.3 (0.04) | 0.8 (0.13) | 182.0 (48.54) |
| **P-M-P** ‡ | 18.1 (38.46) | 71.6 (2.84) | 24.7 (2.02) | 43.9 (6.42) | 164.4 (9.93) | 0.1 (0.07) | 0.3 (0.11) | 0.5 (0.09) | 164.8 (9.95) |
| **L-W-L** § | 50.4 (10.26) | 48.2 (1.62) | 36.9 (8.23) | 72.1 (18.42) | 255.6 (44.95) | 0.1 (0.08) | 0.5 (0.30) | 0.6 (0.30) | 256.2 (45.07) |
| **CP-W-CP** § | 47.2 (5.56) | 99.5 (18.77) | 63.6 (21.70) | 52.6 (2.98) | 262.9 (10.64) | 3.6 (3.31) | 0.6 (0.16) | 4.2 (3.20) | 267.1 (7.89) |
| ***P value*** | 0.212 | 0.741 | 0.166 | 0.653 | 0.194 | 0.595 | 0.549 | 0.495 | 0.173 |
| | **Recovery of $^{13}C$ (%)** | | | | | | | | |
| **P-W-CP** | 7.7 (3.25) | 17.4 (5.08) | 6.4 (2.13) | 12.4 (2.41) | 43.9 (12.60) | 0.3 (0.13) | 8.2 (3.89) ab† | 8.5 (4.01) ab | 52.4 (10.07) a |
| **P-W-P** | 6.7 (2.17) | 17.8 (0.55) | 4.0 (0.21) | 13.9 (4.95) | 42.5 (4.64) | 0.02 (0.004) | 1.1 (0.34) b | 1.1 (0.34) b | 43.6 (4.38) b |
| **P-M-CP** | 8.3 (1.87) | 18.0 (6.30) | 7.1 (2.44) | 11.4 (2.03) | 44.8 (11.97) | 0.1 (0.04) | 8.1 (1.44) ab | 8.2 (1.45) ab | 53.0 (11.55) a |
| **P-M-P** | 6.0 (0.23) | 17.7 (0.70) | 6.1 (0.50) | 10.9 (1.59) | 40.6 (2.46) | 0.04 (0.02) | 2.1 (0.35) b | 2.1 (0.34) b | 42.7 (2.74) b |
| **L-W-L** | 12.5 (2.54) | 11.9 (0.35) | 9.1 (2.03) | 17.8 (4.55) | 51.3 (11.11) | 0.03 (0.02) | 1.6 (0.21) b | 1.6 (0.20) b | 52.9 (11.02) a |
| **CP-W-CP** | 11.7 (1.37) | 24.6 (4.64) | 15.7 (5.37) | 13.0 (0.74) | 65.0 (2.63) | 0.9 (0.82) | 16.6 (4.50) a | 17.5 (4.86) a | 82.5 (4.19) a |
| ***P value*** | 0.212 | 0.740 | 0.166 | 0.654 | 0.194 | 0.595 | 0.006 | 0.006 | 0.028 |
| | **Distribution of Total Recovered Plant $^{13}C$ (%)** | | | | | | | | |
| **P-W-CP** | 16.3 (2.30) | 38.7 (3.07) | 14.4 (2.01) | 29.3 (2.29) | 98.7 (0.56) | 0.8 (0.43) | 0.4 (0.14) | 1.3 (0.56) | 100 |
| **P-W-P** | 16.5 (6.26) | 42.6 (3.27) | 9.6 (0.54) | 31.2 (8.33) | 99.9 (0.04) | 0.04 (0.01) | 0.09 (0.04) | 0.1 (0.04) | 100 |
| **P-M-CP** | 19.0 (1.08) | 37.0 (6.66) | 15.1 (1.55) | 28.4 (6.71) | 99.5 (0.08) | 0.2 (0.04) | 0.2 (0.08) | 0.5 (0.08) | 100 |
| **P-M-P** | 14.8 (1.51) | 43.6 (1.07) | 15.0 (0.50) | 26.4 (2.34) | 99.7 (0.05) | 0.09 (0.05) | 0.2 (0.06) | 0.3 (0.05) | 100 |
| **L-W-L** | 23.6 (0.85) | 25.3 (4.63) | 17.1 (1.57) | 33.7 (5.20) | 99.8 (0.09) | 0.05 (0.03) | 0.2 (0.10) | 0.2 (0.09) | 100 |
| **CP-W-CP** | 17.6 (1.63) | 37.2 (6.96) | 23.9 (8.33) | 19.7 (0.80) | 98.3 (1.30) | 1.4 (1.34) | 0.2 (0.06) | 1.6 (1.30) | 100 |
| ***P value*** | 0.827 | 0.924 | 0.341 | 0.578 | 0.502 | 0.597 | 0.210 | 0.502 | |

† Different letters following means and (standard errors) indicate a significant difference between treatments within each plant part ($P$<0.05) according to Tukey's HSD test.

‡ n=3

§ n=4

¶ Quantity of $^{13}$CdfR determined by multiplying NdfR (%) calculated with the Janzen and Bruinsma equation (1989) and total C (mg core$^{-1}$).

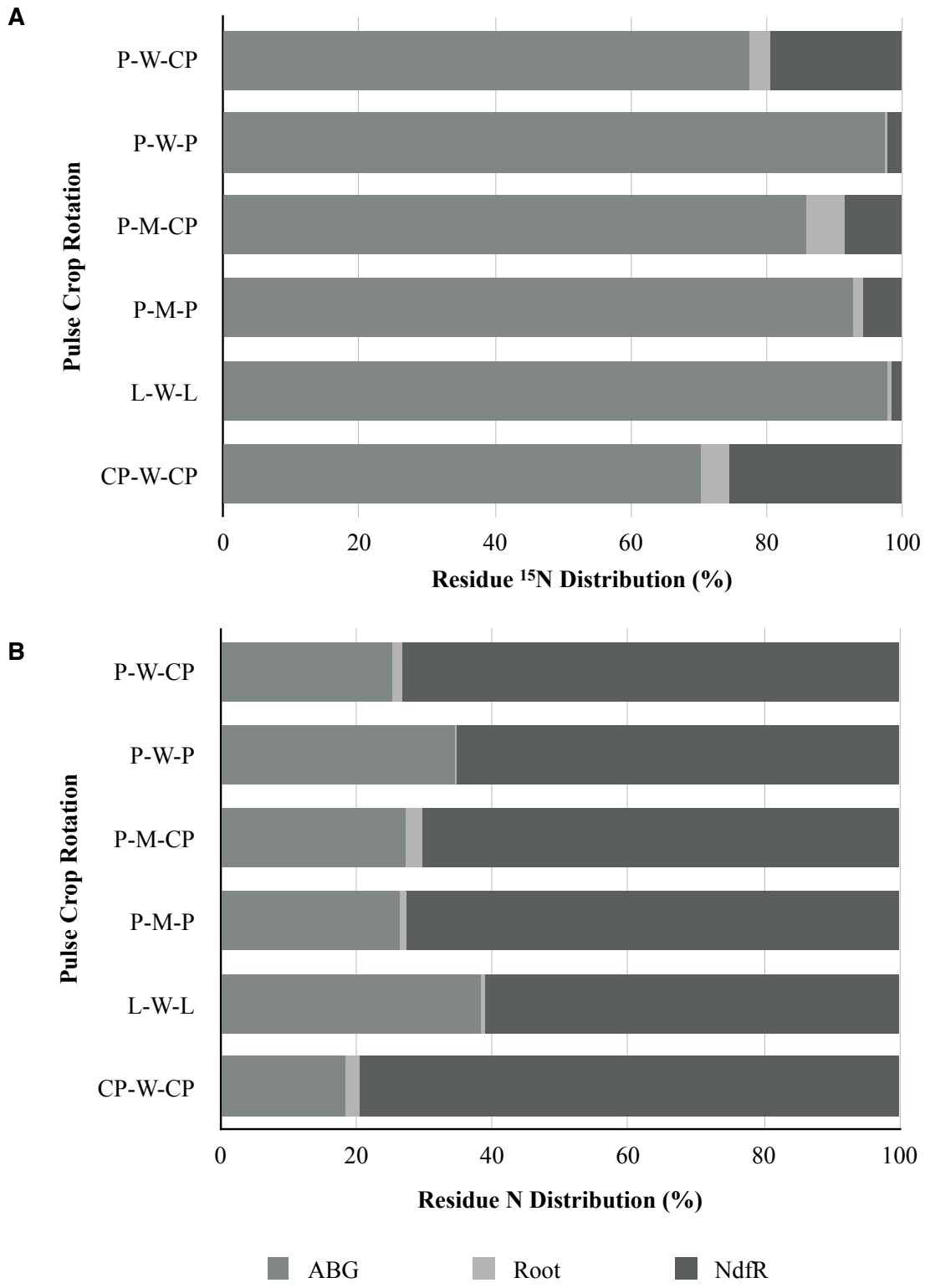


**Figure 4.2. Distribution of ABG residue $^{15}N$, root $^{15}N$ and $^{15}NdfR$ (A) and total ABG residue N, root N and NdfR (B) as a proportion of total residue N in chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}N$-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

**Table 4.7. Plant N (g core$^{-1}$) and the distribution of N (%) as a proportion of total plant N, in the plant parts and bulk rhizodeposition in chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | N (g core$^{-1}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Leaves** | **Seeds** | **Pods** | **Stems** | **ABG Total** | **Rhizodepos-ition¶** | **Roots** | **BG Total** | **Total** |
| **P-W-CP ‡** | 0.1 (0.042) | 0.4 (0.15) | 0.03 (0.011) | 0.1 (0.018) | 0.03 (0.22) | 2.0 (0.89) | 0.03 (0.016) ab† | 2.0 (0.90) | 2.7 (0.68) |
| **P-W-P ‡** | 0.1 (0.061) | 0.6 (0.04) | 0.02 (0.0041) | 0.1 (0.022) | 0.004 (0.069) | 0.7 (0.29) | 0.004 (0.0014) b | 0.7 (0.29) | 1.6 (0.32) |
| **P-M-CP ‡** | 0.1 (0.040) | 0.5 (0.17) | 0.03 (0.0088) | 0.2 (0.023) | 0.03 (0.24) | 1.0 (0.41) | 0.03 (0.0058) a | 1.1 (0.41) | 1.9 (0.23) |
| **P-M-P ‡** | 0.1 (0.021) | 0.3 (0.11) | 0.1 (0.026) | 0.1 (0.052) | 0.01 (0.15) | 0.8 (0.14) | 0.01 (0.0014) b | 0.8 (0.14) | 1.6 (0.16) |
| **L-W-L §** | 0.1 (0.048) | 0.4 (0.01) | 0.1 (0.013) | 0.2 (0.031) | 0.01 (0.22) | 0.7 (0.16) | 0.01 (0.0008) b | 0.7 (0.16) | 1.9 (0.34) |
| **CP-W-CP** | 0.1 (0.039) | 0.7 (0.14) | 0.1 (0.027) | 0.3 (0.014) | 0.1 (0.10) | 2.8 (0.90) | 0.1 (0.018) a | 2.9 (0.92) | 4.0 (0.91) |
| ***P value*** | 0.256 | 0.597 | 0.366 | 0.959 | 0.369 | 0.086 | 0.006 | 0.082 | 0.045 |
| | **Distribution as a proportion of Total Plant N (%)** | | | | | | | | |
| **P-W-CP** | 7.5 (4.52) | 23.6 (15.06) | 1.7 (1.00) | 4.5 (2.24) | 37.2 (22.77) | 61.7 (22.49) | 1.1 (0.40) ab† | 62.8 (22.77) | 100.0 |
| **P-W-P** | 10.5 (5.55) | 39.4 (4.58) | 1.6 (0.36) | 7.7 (0.91) | 59.2 (9.71) | 40.5 (9.72) | 0.3 (0.05) b | 40.8 (9.71) | 100.0 |
| **P-M-CP** | 9.5 (2.76) | 29.2 (11.96) | 1.8 (0.61) | 5.7 (1.11) | 46.2 (16.16) | 52.1 (16.08) | 1.7 (0.11) a | 53.8 (16.16) | 100.0 |
| **P-M-P** | 8.5 (1.30) | 28.9 (7.46) | 3.5 (1.26) | 6.4 (2.56) | 47.4 (7.90) | 52.1 (7.81) | 0.5 (0.09 b | 52.6 (7.90) | 100.0 |
| **L-W-L** | 15.2 (2.19) | 26.7 (3.57) | 4.0 (0.70) | 8.9 (2.23) | 54.8 (5.42) | 44.8 (5.49) | 0.5 (0.11) b | 45.2 (5.42) | 100.0 |
| **CP-W-CP** | 8.3 (2.75) | 18.3 (5.97) | 2.6 (1.25) | 3.4 (1.07) | 32.6 (9.03) | 65.8 (8.97) | 1.6 (0.13) a | 67.4 (9.03) | 100.0 |
| ***P value*** | 0.893 | 0.390 | 0.587 | 0.457 | 0.426 | 0.460 | <0.001 | 0.426 | |

† Different letters following means and (standard errors) indicate a significant difference between treatments within each plant part ($P$<0.05) according to Tukey's HSD test.

‡ n=3

§ n=4

¶ Quantity of NdfR determined by multiplying NdfR (%) calculated with the Janzen and Bruinsma equation (1989) and total N (mg core$^{-1}$).

**Table 4.8. Quantity of plant $^{15}N$ (mg core$^{-1}$), the recovery of $^{15}N$ (%) and the distribution of the recovered $^{15}N$ (%) as a proportion of total plant $^{15}N$, in the plant parts and bulk rhizodeposition in pulse crop rotations grown in a greenhouse and supplied with $^{15}N$-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | $^{15}N$ (mg core$^{-1}$) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Leaves** | **Seeds** | **Pods** | **Stems** | **ABG Total** | **Rhizode-position¶** | **Roots** | **BG Total** | **ABG +BG Total** |
| **P-W-CP ‡** | 3.7 (1.40) | 11.1 (5.88) | 0.9 (0.37) | 2.3 (0.91) | 18.1 (7.94) | 1.3 (0.72) | 0.2 (0.08) b† | 1.5 (0.08) ab | 19.6 (0.13) |
| **P-W-P ‡** | 5.6 (2.19) | 22.6 (1.45) | 1.0 (0.17) | 5.6 (1.45) | 34.8 (1.86) | 0.3 (0.18) | 0.04 (0.01) b | 0.3 (0.01) ab | 35.1 (0.06) |
| **P-M-CP ‡** | 4.0 (0.83) | 10.4 (3.61) | 0.7 (0.17) | 2.2 (0.40) | 17.2 (4.83) | 0.6 (0.32) | 0.4 (0.10) ab | 1.0 (0.10) ab | 18.2 (0.04) |
| **P-M-P ‡** | 3.3 (0.38) | 14.3 (3.60) | 2.0 (0.91) | 2.7 (1.17) | 22.4 (5.07) | 0.5 (0.21) | 0.1 (0.04 ) b | 0.6 (0.04) ab | 23.0 (0.11) |
| **L-W-L §** | 10.6 (2.79) | 16.9 (2.24) | 1.6 (0.42) | 3.5 (1.15) | 51.5 (13.6) | 0.2 (0.08) | 0.1 (0.01) b | 0.3 (0.01) b | 51.8 (0.30) |
| **CP-W-CP §** | 5.3 (0.85) | 13.3 (2.75) | 2. (1.27) | 2.8 (0.74) | 23.5 (0.62) | 3.7 (1.67) | 0.6 (0.14) a | 4.3 (0.14) a | 27.8 (0.16) |
| ***P value*** | 0.077 | 0.061 | 0.675 | 0.221 | 0.047 | 0.074 | 0.002 | 0.041 | 0.058 |
| | **Recovery of $^{15}N$ (%)** | | | | | | | | |
| **P-W-CP** | 8.8 (3.31) | 26.2 (12.61) | 2.2 (0.87) | 5.5 (2.15) | 42.7 (18.71) | 3.1 (1.69) | 0.5 (0.18) b | 3.6 (1.81) ab | 46.3 (17.07) |
| **P-W-P** | 13.1 (5.16) | 53.4 (3.41) | 2.3 (0.40) | 13.3 (2.39) | 82.1 (4.38) | 0.6 (0.42) | 0.1 (0.03) b | 0.7 (0.44) ab | 82.8 (4.62) |
| **P-M-CP** | 9.4 (1.96) | 24.5 (8.52) | 1.6 (0.41) | 5.1 (0.95) | 40.6 (11.38) | 1.3 (0.76) | 1.0 (0.24) ab | 2.3 (0.70) ab | 43.0 (10.70) |
| **P-M-P** | 7.9 (0.90) | 33.6 (8.49) | 4.8 (2.14) | 6.4 (2.75) | 52.7 (11.96) | 1.2 (0.49) | 0.3 (0.08) b | 1.5 (0.3058 ab | 54.2 (11.73) |
| **L-W-L** | 25.0 (6.57) | 39.6 (5.29) | 3.7 (0.99) | 8.2 (2.70) | 76.5 (14.45) | 0.5 (0.19) | 0.1 (0.03) b | 0.7 (0.17) b | 77.8 (14.51) |
| **CP-W-CP** | 12.5 (1.99) | 31.2 (6.49) | 5.0 (2.99) | 6.6 (1.75) | 55.4 (1.45) | 8.8 (3.93) | 1.4 (0.33) a | 10.2 (4.20) a | 65.5 (3.63) |
| ***P value*** | 0.077 | 0.061 | 0.675 | 0.221 | 0.047 | 0.074 | 0.002 | 0.051 | 0.058 |
| | **Distribution of Total Recovered Plant $^{15}N$ (%)** | | | | | | | | |
| **P-W-CP** | 19.2 (1.97) | 52.3 (5.92) | 4.7 (1.73) | 11.8 (0.21) | 88.0 (5.98) | 10.4 (5.39) | 1.6 (0.80) ab | 12.0 (5.98) | 100 |
| **P-W-P** | 15.7 (5.87) | 64.5 (2.71) | 2.7 (0.36) | 16.2 (3.14) | 99.2 (0.48) | 0.7 (0.46) | 0.1 (0.02) b | 0.8 (0.48) | 100 |
| **P-M-CP** | 22.7 (1.49) | 52.6 (8.92) | 3.6 (0.09) | 13.3 (3.12) | 92.2 (4.70) | 5.1 (4.05) | 2.6 (0.77) a | 7.8 (4.70) | 100 |
| **P-M-P** | 16.5 (4.90) | 61.0 (5.78) | 8.4 (2.53) | 10.8 (2.38) | 96.8 (1.77) | 2.6 (1.49) | 0.6 (0.29) ab | 3.2 (1.77) | 100 |
| **L-W-L** | 30.1 (2.62) | 54.7 (5.87) | 4.4 (0.54) | 9.9 (2.07) | 99.1 (0.17) | 0.7 (0.12) | 0.2 (0.09) b | 0.9 (0.17) | 100 |
| **CP-W-CP** | 19. (3.72) | 47.3 (9.50) | 8.1 (4.95) | 10.4 (3.07) | 85.4 (5.70) | 12.6 (5.36) | 2.1 (0.44) ab | 14.6 (5.17) | 100 |
| ***P value*** | 0.793 | 0.260 | 0.525 | 0.296 | 0.099 | 0.129 | 0.008 | 0.099 | - |

† Different letters following means and (standard errors) indicate a significant difference between treatments within each plant part ($P$<0.05) according to Tukey's HSD test.

‡ n=3

§ n=4

¶ Quantity of NdfR determined by multiplying NdfR (%) calculated with the Janzen and Bruinsma equation (1989) and total $^{15}N$ (mg core$^{-1}$)

**Table 4.9. The C:N ratios of the bulk soil, HF, LF,VLF and water extractable OM and the SMB pool in each soil core where chickpea, lentil or pea were grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | C:N Ratios | | | | |
|---|---|---|---|---|---|
| | **Bulk Soil** | **HF** | **LF** | **VLF** | **WEOM** |
| **P-W-CP** ‡ | 8.7 (0.05) b† | 8.7 (0.03) | 11.2 (0.79) | 20.2 (0.48) | 7.0 (4.11) |
| **P-W-P** ‡ | 9.0 (0.07) ab | 8.8 (0.03) | 11.4 (0.32) | 15.0 (3.63) | 2.5 (1.86) |
| **P-M-CP** ‡ | 8.9 (0.11) ab | 8.7 (0.05) | 9.8 (0.29) | 19.1 (1.80) | 2.8 (1.01) |
| **P-M-P** ‡ | 8.6 (0.01) b | 8.8 (0.07) | 11.1 (0.61 | 19.5 (2.35) | 0.4 (0.05) |
| **L-W-L** § | 8.7 (0.10) b | 8.6 (0.03) | 10.2 (1.37) | 20.6 (0.65) | 4.2 (2.65) |
| **CP-W-CP** § | 9.2 (0.12) a | 8.9 (0.10) | 11.0 (0.23) | 20.7 (2.74) | 11.6 (8.48) |
| ***P value*** | 0.005 | 0.117 | 0.735 | 0.511 | 0.602 |

† Different letters following means and (standard errors) indicate a significant difference between treatments within each plant part ($P$<0.05) according to Tukey's HSD test.
‡ n=3
§ n=4

**Table 4.10. The proportion (%) of the HF, LF, and VLF SOM in the bulk soil, on a mass basis, and the %C and %N in the HF, LF and VLF SOM matter in chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | **HF** | | | **LF** | | | **VLF** | | |
|---|---|---|---|---|---|---|---|---|---|
| | %Mass | %C | %N | %Mass | %C | %N | %Mass | %C | %N |
| **P-W-CP** ‡ | 77.3 (4.23) | 1.2 (0.03) | 0.1 (0.003) | 3.6 (2.73) | 3.5 (1.10) | 0.3 (0.08) | 0.1 (0.04) | 26.6 (2.19) | 1.3 (0.08) |
| **P-W-P** ‡ | 57.4 (23.92) | 1.2 (0.07) | 0.1 (0.008) | 3.0 (0.83) | 2.9 (0.47) | 0.3 (0.03) | 0.1 (0.05) | 14.1 (5.95) | 0.8 (0.25) |
| **P-M-CP** ‡ | 69.4 (4.66) | 1.2 (0.07) | 0.1 (0.008) | 5.5 (1.44) | 1.7 (0.38) | 0.2 (0.03) | 0.1 (0.02) | 26.2 (6.88) | 1.3 (0.27) |
| **P-M-P** ‡ | 85.9 (6.59) | 1.1 (0.04) | 0.1 (0.005) | 1.8 (0.49) | 3.3 (1.33) | 0.3 (0.10) | 0.04 (0.01) | 21.5 (6.62) | 1.0 (0.24) |
| **L-W-L** § | 80.3 (1.79) | 1.1 (0.04) | 0.1 (0.005) | 2.8 (0.99) | 2.3 (0.76) | 0.2 (0.06) | 0.04 (0.02) | 26.3 (3.27) | 1.3 (0.16) |
| **CP-W-CP**§ | 74.4 (3.33) | 1.2 (0.02) | 0.1 (0.002) | 5.0 (0.80) | 2.8 (0.17) | 0.3 (0.01) | 0.1 (0.02) | 24.3 (2.11) | 1.2 (0.12) |
| ***p-value*** | 0.440 | 0.283 | 0.363 | 0.392 | 0.647 | 0.636 | 0.328 | 0.433 | 0.462 |

‡ n=3
§ n=4

**Table 4.11. The proportion (%) of the WEOM in the bulk soil, on a mass basis, and the %C and %N in the WEOM matter in chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | WEOM | | |
|---|---|---|---|
| | %Mass | %C | %N |
| **P-W-CP** ‡ | 0.04 (0.02) | 1.9 (0.90) | 0.7 (0.48) |
| **P-W-P** ‡ | 0.1 (0.001) | 1.1 (0.45) | 1.3 (0.99) |
| **P-M-CP** ‡ | 0.1 (0.01) | 1.0 (0.45) | 0.3 (0.04) |
| **P-M-P** ‡ | 0.1 (0.01) | 0.7 (0.09) | 2.0 (0.43) |
| **L-W-L** § | 0.1 (0.01) | 0.7 (0.28) | 0.3 (0.13) |
| **CP-W-CP**§ | 0.04 (0.01) | 2.1 (1.29) | 0.3 (0.14) |
| ***p-value*** | 0.517 | 0.702 | 0.093 |

‡ n=3
§ n=4

**Table 4.12. Carbon (g core$^{-1}$) and C distribution (%), as a proportion of total soil C, in the bulk soil, the HF, LF and VLF organic matter, and in the SMB pool in chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | C (g core$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | **Bulk Soil** | **HF** | **LF** | **VLF** | **WEOM** |
| **P-W-CP** ‡ | 204.4 (19.25) | 136.2 (4.33) | 11.0 (6.05) | 3.6 (2.11) | 0.1 (0.03) |
| **P-W-P** ‡ | 214.3 (5.91) | 107.0 (45.15) | 13.4 (3.02) | 2.9 (2.11) | 0.1 (0.04) |
| **P-M-CP** ‡ | 202.9 (8.94) | 123.4 (9.11) | 14.3 (6.27) | 3.2 (1.20) | 0.1 (0.04) |
| **P-M-P** ‡ | 178.8 (8.92) | 139.9 (7.81) | 7.7 (1.87) | 1.2 (0.41) | 0.1 (0.01) |
| **L-W-L** § | 172.7 (10.62) | 131.8 (7.20) | 6.7 (2.60) | 1.5 (0.53) | 0.1 (0.02) |
| **CP-W-CP** § | 218.2 (9.20) | 141.9 (4.93) | 21.3 (1.95) | 2.6 (0.83) | 0.1 (0.04) |
| ***P-value*** | 0.045 | 0.747 | 0.106 | 0.761 | 0.949 |
| | **Distribution of total soil C (%)** | | | | |
| **P-W-CP** | 100.0 (0.00) | 67.5 (4.57) | 5.1 (2.45) | 1.7 (0.86) | 0.05 (0.02) |
| **P-W-P** | 100.0 (0.00) | 51.2 (21.91) | 6.2 (1.42) | 1.4 (1.01) | 0.05 (0.02) |
| **P-M-CP** | 100.0 (0.00) | 60.7 (2.19) | 4.9 (2.5678 | 1.5 (0.57) | 0.04 (0.02) |
| **P-M-P** | 100.0 (0.00) | 78.4 (3.78) | 4.4 (1.22) | 0.7 (0.21) | 0.04 (0.01) |
| **L-W-L** | 100.0 (0.00) | 76.6 (3.12) | 3.7 (1.28) | 0.9 (0.30) | 0.04 (0.01) |
| **CP-W-CP** | 100.0 (0.00) | 65.5 (3.90) | 9.9 (1.26) | 1.2 (0.37) | 0.04 (0.02) |
| ***P-value*** | | 0.747 | 0.163 | 0.844 | 0.990 |

‡ n=3
§ n=4

**Table 4.13. Quantity of $^{13}C$ (mg core$^{-1}$) the recovery of $^{13}C$ (%) and the distribution of the recovered $^{13}C$ (%), as a proportion of total soil $^{13}C$, in the bulk soil, in the HF, LF, VLF and WEOM, the SMB and inorganic N pools in chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}N$-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | $^{13}C$ (mg core$^{-1}$) | | | | |
|---|---|---|---|---|---|
| | **Bulk Soil** | **HF** | **LF** | **VLF** | **WEOM** |
| **P-W-CP** ‡ | 15.5 (7.30) | 7.1 (3.32) | 1.6 (0.90) ab† | 0.5 (0.37) | 0.002 (0.0013) |
| **P-W-P** ‡ | 2.4 (0.31) | 5.7 (5.09) | 0.6 (0.09) b | 0.1 (0.08) | 0.004 (0.0020) |
| **P-M-CP** ‡ | 8.5 (1.40) | 4.4 (1.56) | 1.5 (0.45) ab | 0.4 (0.12) | 0.007 (0.0025) |
| **P-M-P** ‡ | 2.7 (1.69) | 1.3 (0.17) | 0.4 (0.12) b | 0.0 (0.03) | 0.002 (0.0005) |
| **L-W-L** § | 3.6 (1.26) | 1.9 (0.27) | 0.4 (0.11) b | 0.2 (0.09) | 0.002 (0.0003) |
| **CP-W-CP** § | 14.0 (5.76) | 6.3 (0.85) | 3.3 (0.38) a | 0.1 (0.07) | 0.006 (0.0024) |
| ***P-value*** | 0.130 | 0.416 | 0.002 | 0.427 | 0.284 |
| | **Recovery of $^{13}C$ (%)** | | | | |
| **P-W-CP** | 3.8 (1.80) | 1.7 (0.82) | 0.4 (0.22) a | 0.1 (0.09) | 0.001 (0.0003) |
| **P-W-P** | 0.6 (0.08) | 1.4 (1.26) | 0.1 (0.02) b | 0.03 (0.02) | 0.001 (0.0005) |
| **P-M-CP** | 2.1 (0.35) | 1.1 (0.38) | 0.4 (0.11) ab | 0.1 (0.03) | 0.002 (0.006) |
| **P-M-P** | 0.7 (0.40) | 0.3 (0.04) | 0.1 (0.03) b | 0.01 (0.01) | 0.0004 (0.0001) |
| **L-W-L** | 0.9 (0.31) | 0.5 (0.07) | 0.1 (0.03) b | 0.04 (0.02) | 0.0005 (0.00008) |
| **CP-W-CP** | 3.5 (1.42) | 1.5 (0.21) | 0.8 (0.09) a | 0.04 (0.02) | 0.001 (0.0006) |
| ***P-value*** | 0.130 | 0.416 | 0.002 | 0.427 | 0.284 |
| | **Distribution as a proportion of total soil $^{13}C$ (%)** | | | | |
| **P-W-CP** | 100.0 (0.00) | 62.2 (19.95) | 21.3 (12.16) | 5.0 (2.57) | 0.2 (0.16) |
| **P-W-P** | 100.0 (0.00) | 49.5 (27.03) | 28.6 (9.06) | 5.7 (4.51) | 0.2 (0.12) |
| **P-M-CP** | 100.0 (0.00) | 52.9 (18.79) | 17.2 (5.17) | 5.4 (2.24) | 0.1 (0.03) |
| **P-M-P** | 100.0 (0.00) | 33.5 (13.69) | 14.3 (10.17) | 0.3 (0.01) | 0.04 (0.006) |
| **L-W-L** | 100.0 (0.00) | 63.8 (15.60) | 14.3 (3.54) | 5.4 (3.57) | 0.1 (0.02) |
| **CP-W-CP** | 100.0 (0.00) | 64.1 (17.93) | 37.3 (12.51) | 1.5 (0.84) | 0.1 (0.07) |
| ***P-value*** | | 0.650 | 0.454 | 0.723 | 0.879 |

† Different letters following means and (standard errors) indicate a significant difference between treatments within each plant part ($P$<0.05) according to Tukey HSD.

‡ n=3

§ n=4

**Table 4.14. Nitrogen (g core$^{-1}$) and N distribution (%), as a proportion of total soil N, in the bulk soil, the HF, LF, VLF and water extractable organic matter, and in the SMB pool in chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | N (g core$^{-1}$) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Bulk Soil** | **HF** | **LF** | **VLF** | **WEOM** | **$NH_4^+$** | **$NO_3$** | **SMB** |
| **P-W-CP** ‡ | 23.5 (2.29) | 15.6 (0.50) | 1.1 (0.65) | 0.2 (0.10) | 0.1 (0.05) | 0.1 (0.03) | 0.2 (0.074) | 0.002 (0.0006) |
| **P-W-P** ‡ | 23.9 (0.84) | 12.2 (5.11) | 1.2 (0.27) | 0.2 (0.09) | 0.1 (0.09) | 0.03 (0.003) | 0.2 (0.093) | 0.002 (0.0006) |
| **P-M-CP** ‡ | 22.7 (1.09) | 14.1 (0.98) | 1.4 (0.60) | 0.2 (0.05) | 0.02 (0.004) | 0.1 (0.06) | 0.2 (0.071) | 0.002 (0.0001) |
| **P-M-P** ‡ | 20.7 (1.03) | 15.9 (0.78) | 0.7 (0.16) | 0.1 (0.02) | 0.2 (0.05) | 0.03 (0.003) | 0.4 (0.11) | 0.001 (0.0006) |
| **L-W-L** § | 19.9 (1.04) | 15.3 (0.84) | 0.6 (0.24) | 0.1 (0.03) | 0.03 (0.01) | 0.04 (0.002) | 0.2 (0.021) | 0.005 (0.003) |
| **CP-W-CP** § | 23.7 (0.71) | 16.0 (0.52) | 2.0 (0.21) | 0.1 (0.04) | 0.03 (0.01) | 0.1 (0.02) | 0.1 (0.011) | 0.002 (0.0007) |
| ***P-value*** | 0.121 | 0.752 | 0.151 | 0.655 | 0.077 | 0.291 | 0.065 | 0.678 |
| | **Distribution as a proportion of total soil N (%)** | | | | | | | |
| **P-W-CP** | 100.0 (0.00) | 67.1 (3.80) | 4.3 (2.34) | 0.7 (0.36) | 0.3 (0.29) ab† | 0.2 (0.12) | 0.8 (0.42) ab | 7.1 (2.17) |
| **P-W-P** | 100.0 (0.00) | 52.5 (21.60) | 5.0 (1.18) | 0.7 (0.40) | 0.5 (0.35) ab | 0.1 (0.01) | 1.0 (0.35) ab | 9.0 (2.56) |
| **P-M-CP** | 100.0 (0.00) | 62.2 (2.05) | 6.2 (2.40) | 0.7 (0.18) | 0.1 (0.02) ab | 0.5 (0.28) | 0.7 (0.28 ab | 9.3 (0.36) |
| **P-M-P** | 100.0 (0.00) | 77.1 (3.55) | 3.4 (0.94) | 0.3 (0.06) | 1.0 (0.22) a | 0.1 (0.01) | 1.9 (0.47) a | 7.4 (3.24) |
| **L-W-L** | 100.0 (0.00) | 77.0 (2.39) | 3.1 (1.07) | 0.4 (0.14) | 0.1 (0.05) ab | 0.2 (0.02) | 0.8 (0.07) ab | 24.0 (15.50) |
| **CP-W-CP** | 100.0 (0.00) | 67.6 (3.36) | 8.3 (1.10) | 0.5 (0.15) | 0.1 (0.06) b | 0.4 (0.08) | 0.4 (0.06) b | 7.2 (2.85) |
| ***P-value*** | | 0.404 | 0.166 | 0.734 | 0.049 | 0.347 | 0.040 | 0.595 |

† Different letters following means and (standard errors) indicate a significant difference between treatments within each plant part ($P$<0.05) according to Tukey's HSD test.

‡ n=3

§ n=4

**Table 4.15. Quantity of $^{15}N$ (mg core$^{-1}$) the recovery of $^{15}N$ (%) and the distribution of the recovered $^{15}N$ (%), as a proportion of total soil N, in the bulk soil, in the HF, LF, VLF and water extractable organic matter, and in the SMB and inorganic N pools in chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}N$-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | $^{15}N$ (mg core$^{-1}$) | | | | | $^{15}N$ (µg core$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|
| | **Bulk Soil** | **HF** | **LF** | **VLF** | **WEOM** | **$NH_4^+$** | **$NO_3^-$** | **SMB** |
| **P-W-CP** ‡ | 12.3 (5.42) | 1.9 (0.65) | 0.6 (0.22) | 0.1 (0.11) | 0.1 (0.040) b† | 0.1 (0.05) ab | 0.5 (0.25) | 4.4 (1.6) |
| **P-W-P** ‡ | 7.0 (2.35) | 2.0 (1.47) | 0.7 (308.62) | 0.3 (0.18) | 0.3 (0.15) a | 0.01 (0.001) b | 0.08 (0.023) | 10.2 (4.2) |
| **P-M-CP** ‡ | 11.5 (2.62) | 3.7 (1.37) | 1.6 (0.93) | 0.2 (0.13) | 0.1 (0.013) b | 0.1 (0.059) ab | 0.4 (0.34) | 10.3 (1.8) |
| **P-M-P** ‡ | 11.7 (3.50) | 3.3 (0.35) | 0.5 (0.13) | 0.04 (0.0052) | 0.5 (0.16) a | 0.03 (0.011) b | 0.3 (0.060) | 3.1 (1.2) |
| **L-W-L** § | 6.0 (1.03) | 2.1 (0.09) | 0.5 (0.21) | 0.1 (0.031) | 0.1 (0.034) b | 0.04 (0.014) b | 0.2 (0.055) | 20.4 (1.1) |
| **CP-W-CP** § | 25.6 (7.51) | 3.6 (0.79) | 1.5 (0.18) | 0.1 (0.022) | 0.1 (0.032) b | 0.2 (0.063) a | 0.2 (0.036) | 5.4 (1.6) |
| ***P-value*** | 0.071 | 0.447 | 0.193 | 0.470 | 0.021 | 0.013 | 0.577 | 0.573 |
| | **Recovery of $^{15}N$ (%)** | | | | | | | |
| **P-W-CP** | 14.9 (6.57) | 2.3 (0.79) | 0.7 (0.27) | 0.1 (0.13) | 0.1 (0.05) b | 0.1 (0.06) ab | 0.6 (0.30) | 0.2 (0.10) |
| **P-W-P** | 8.5 (2.85) | 2.4 (1.78) | 0.8 (0.37) | 0.3 (0.22) | 0.3 (0.19) ab | 0.01 (0.002) b | 0.1 (0.03) | 0.6 (0.24) |
| **P-M-CP** | 14.0 (3.18) | 4.5 (1.67) | 1.9 (1.13) | 0.3 (0.16) | 0.1 (0.02) b | 0.2 (0.07) ab | 0.5 (0.41) | 0.4 (0.11) |
| **P-M-P** | 14.2 (4.24) | 4.0 (0.43) | 0.6 (0.16) | 0.0 (0.01) | 0.6 (0.20) a | 0.03 (0.01) b | 0.4 (0.07) | 0.2 (0.07) |
| **L-W-L** | 7.3 (1.25) | 2.5 (0.11) | 0.6 (0.25) | 0.1 (0.04) | 0.1 (0.04) b | 0.1 (0.02) b | 0.2 (0.07) | 1.0 (0.69) |
| **CP-W-CP** | 31.0 (9.10) | 4.4 (0.95) | 1.8 (0.22) | 0.1 (0.03) | 0.1 (0.04) b | 0.3 (0.08) a | 0.3 (0.04) | 0.3 (0.09) |
| ***P-value*** | 0.071 | 0.447 | 0.193 | 0.470 | 0.021 | 0.013 | 0.577 | 0.573 |
| | **Distribution of total soil $^{15}N$ (%)** | | | | | | | |
| **P-W-CP** | 100.0 (0.00) | 20.3 (5.88) | 6.4 (1.74) | 1.0 (0.68) | 2.3 (2.18) | 0.002 (0.0007) | 0.02 (0.01) | 0.05 (0.02) |
| **P-W-P** | 100.0 (0.00) | 24.7 (11.77) | 8.7 (1.25) | 3.0 (1.24) | 4.6 (2.36) | 0.0002 (0.00004) | 0.001 (0.0001) | 0.2 (0.13) |
| **P-M-CP** | 100.0 (0.00) | 34.5 (11.66) | 14.8 (7.65) | 2.0 (1.10) | 0.5 (0.17) | 0.001 (0.0007) | 0.004 (0.003) | 0.1 (0.02) |
| **P-M-P** | 100.0 (0.00) | 34.4 (10.69) | 4.7 (1.40) | 0.4 (0.06) | 4.6 (1.51) | 0.0002 (0.0001) | 0.003 (0.0003) | 0.04 (0.02) |
| **L-W-L** | 100.0 (0.00) | 37.2 (5.02) | 7.3 (2.09) | 1.4 (0.29) | 1.0 (0.52) | 0.0009 (0.0003) | 0.003 (0.001) | 0.3 (0.18) |
| **CP-W-CP** | 100.0 (0.00) | 23.9 (11.28) | 12.1 (7.64) | 0.3 (0.14) | 0.9 (0.80) | 0.002 (0.002) | 0.002 (0.001) | 0.03 (0.02) |
| ***P-value*** | | 0.748 | 0.729 | 0.093 | 0.199 | 0.600 | 0.480 | 0.388 |

† Different letters following means and (standard errors) indicate a significant difference between treatments within each plant part ($P<0.05$) according to Tukey HSD.

‡ n=3

§ n=4

**Table 4.16. The C:N ratios of rhizodeposition in the bulk soil, HF, LF,VLF and water extractable organic matter and the SMB pool in chickpea, lentil, or pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | C:N Ratios | | | | |
|---|---|---|---|---|---|
| | **Bulk** | **HF** | **LF** | **VLF** | **WEOM** |
| **P-W-CP** ‡ | 5.1 (0.60) | 18.0 (4.26) | 13.6 (4.32) | 34.9 (13.91) | 0.6 (0.39) |
| **P-W-P** ‡ | 9.7 (1.86) | 44.2 (22.11) | 29.2 (18.03) | 35.5 (34.70) | 0.3 (0.07) |
| **P-M-CP** ‡ | 12.6 (4.87) | 18.9 (7.80) | 15.6 (2.06) | 55.8 (36.94) | 4.5 (3.90) |
| **P-M-P** ‡ | 8.6 (4.03) | 10.1 (3.71) | 13.2 (7.64) | 21.8 (17.02) | 0.1 (0.02) |
| **L-W-L** § | 5.5 (1.36) | 8.3 (1.45) | 12.1 (4.06) | 37.6 (30.38) | 1.3 (1.05) |
| **CP-W-CP** § | 8.5 (4.68) | 25.4 (13.78) | 32.9 (18.92) | 45.2 (29.08) | 1.0 (0.48) |
| ***P value*** | 0.683 | 0.368 | 0.674 | 0.977 | 0.541 |

‡ n=3
§ n=4

**Table 4.17. CdfR (mg core$^{-1}$) and distribution of CdfR (%), as a proportion of plant C, in the bulk soil, in the HF, LF and VLF organic matter, and in the SMB pool in chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | CdfR (mg core$^{-1}$) ¶ | | | | |
|---|---|---|---|---|---|
| | **Bulk Soil** | **HF** | **LF** | **VLF** | **WEOM** |
| **P-W-CP** ‡ | 10891.7 (4997.85) | 4928.0 (1931.20) | 1204.2 (689.04) | 357.7 (286.01) | 2.1 (1.42) |
| **P-W-P** ‡ | 5676.8 (1253.90) | 16567.7 (15627.53) | 1810.6 (257.92) | 432.4 (374.20) | 9.8 (6.54) |
| **P-M-CP** ‡ | 9052.5 (1471.00) | 4674.1 (1544.96) | 1541.7 (446.99) | 421.5 (130.98) | 6.9 (2.51) |
| **P-M-P** ‡ | 5983.8 (1830.45) | 2069.6 (155.90) | 326.8 (171.78) | 61.3 (47.74) | 3.4 (0.90) |
| **L-W-L** § | 4005.7 (1832.65) | 1891.2 (342.94) | 475.1 (169.97) | 182.1 (91.74) | 1.9 (0.33) |
| **CP-W-CP** § | 30377.9 (23759.79) | 7782.5 (2440.74) | 4557.3 (1941.84) | 326.7 (260.84) | 5.9 (1.77) |
| ***P value*** | 0.603 | 0.603 | 0.100 | 0.896 | 0.385 |
| | **Distribution of CdfR as a proportion of plant C (%)** | | | | |
| **P-W-CP** | 40.9 (18.28) | 19.2 (7.89) | 4.4 (2.16) ab† | 1.2 (0.94) | 0.01 (0.0064) |
| **P-W-P** | 28.5 (5.10) | 81.2 (75.80) | 8.4 (0.88) a | 2.0 (1.67) | 0.05 (0.029) |
| **P-M-CP** | 38.3 (4.26) | 19.1 (5.13) | 6.2 (1.32) ab | 2.2 (1.13) | 0.03 (0.010) |
| **P-M-P** | 29.2 (7.39) | 10.0 (1.26) | 1.6 (0.86) b | 0.5 (0.33) | 0.02 (0.0035) |
| **L-W-L** | 18.6 (6.97) | 10.1 (3.09) | 2.2 (0.62) b | 1.1 (0.64) | 0.01 (0.0006) |
| **CP-W-CP** | 37.3 (15.85) | 16.7 (1.55) | 8.8 (0.94) a | 0.4 (0.19) | 0.02 (0.0080) |
| ***P value*** | 0.622 | 0.542 | 0.002 | 0.477 | 0.302 |

† Different letters following means and (standard errors) indicate a significant difference between treatments within each plant part ($P<0.05$) according to Tukey's HSD test.

‡ n=3

§ n=4

¶ Quantity of CdfR determined by multiplying CdfR (%) calculated with the Janzen and Bruinsma equation (1989) and total C (mg core$^{-1}$) in the corresponding soil pool.

**Table 4.18. NdfR (mg core$^{-1}$) and distribution of NdfR (%), as a proportion of plant N, in the bulk soil, in the HF, LF, VLF and water extractable organic matter, the SMB and inorganic N pools in chickpea, lentil and pea grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of three or four cores and (standard errors).**

| | NdfR (mg core$^{-1}$) ¶ | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Bulk** | **HF** | **LF** | **VLF** | **WEOM** | **$NH_4^+$** | **$NO_3$** | **SMB** |
| **P-W-CP** ‡ | 1991.0 (885.18) | 306.6 (132.84) | 100.4 (45.39) | 22.0 (20.35) | 6.6 (4.46) | 18.1 (10.46) | 59.2 (25.35) | 1.7 (0.55) |
| **P-W-P** ‡ | 695.8 (288.15) | 212.1 (165.77) | 67.6 (36.43) | 28.2 (20.41) | 25.8 (12.87) | 1.0 (0.17) | 7.9 (2.87) | 2.1 (0.56) |
| **P-M-CP** ‡ | 1030.0 (406.31) | 262.1 (25.98) | 94.7 (17.19) | 12.3 (3.67) | 5.5 (2.30) | 10.2 (5.94) | 18.2 (9.55) | 2.1 (0.11) |
| **P-M-P** ‡ | 814.0 (141.78) | 249.7 (44.27) | 35.3 (9.62) | 2.8 (0.03) | 35.4 (9.36) | 1.9 (0.63) | 21.0 (1.50) | 1.5 (0.63) |
| **L-W-L** § | 681.8 (158.19) | 231.7 (22.92) | 58.9 (27.74) | 10.6 (4.23) | 7.3 (3.96) | 4.8 (1.54) | 18.9 (6.66) | 4.9 (3.23) |
| **CP-W-CP** § | 2834.3 (901.49) | 443.9 (124.61) | 183.6 (49.61) | 5.3 (1.71) | 10.9 (6.42) | 34.3 (13.68) | 31.0 (10.22) | 1.7 (0.66) |
| ***P-value*** | 0.086 | 0.576 | 0.105 | 0.571 | 0.055 | 0.068 | 0.116 | 0.526 |
| | **Distribution of NdfR as a proportion of plant N (%)** | | | | | | | |
| **P-W-CP** | 61.7 (22.49) | 10.1 (3.16) | 3.3 (1.12) | 0.7 (0.6) | 0.4 (0.35) | 0.6 (0.28) | 3.3 (2.30) | 0.1 (0.019) |
| **P-W-P** | 40.5 (9.72) | 10.7 (7.03) | 3.7 (1.36) | 1.4 (0.84) | 1.8 (1.05) | 0.06 (0.01) | 0.5 (0.09) | 0.1 (0.036) |
| **P-M-CP** | 52.1 (16.08) | 14.3 (1.27) | 5.5 (1.64) | 0.7 (0.27) | 0.3 (0.10) | 0.6 (0.29) | 1.2 (0.75) | 0.1 (0.016) |
| **P-M-P** | 52.1 (7.81) | 16.5 (3.86) | 2.2 (0.46) | 0.2 (0.02) | 2.4 (0.75) | 0.1 (0.04) | 1.4 (0.12) | 0.1 (0.045) |
| **L-W-L** | 44.8 (3.95) | 16.1 (1.75) | 3.5 (1.37) | 0.6 (0.20) | 0.5 (0.23) | 0.4 (0.14) | 1.3 (0.45) | 0.3 (0.19) |
| **CP-W-CP** | 65.8 (8.97) | 12.8 (3.98) | 6.0 (2.77) | 0.2 (0.06) | 0.4 (0.31) | 1.1 (0.67) | 1.0 (0.53) | 0.1 (0.022) |
| ***P-value*** | 0.460 | 0.885 | 0.561 | 0.294 | 0.054 | 0.393 | 0.463 | 0.288 |

‡ n=3

§ n=4

¶ Quantity of NdfR determined by multiplying NdfR (%) calculated with the Janzen and Bruinsma equation (1989) and total N (mg core$^{-1}$) in the corresponding soil pool.

## 4.5 Discussion

### 4.5.1 Aboveground and belowground partitioning of C and N

#### *4.5.1.1 Carbon*

The ABG and BG partitioning of C did not differ between pulse crop rotations. In pea the proportion of BG plant C (30.9%) agrees with Wichern et al. (2007a), where 33.7% of mature plant C was from BG contributions. When seed is excluded from the budget, ABG residue C is still greater than BG residue C (43%) in the pea rotations. Similarly, ABG residue C in L-W-L (75%) and CP-W-CP (52%) made up a greater proportion of residue C than BG-C (25% and 48%, respectively). The two chickpea rotations where pea was the previously grown pulse crop (P-M-CP and P-W-CP) do not follow this trend as BG-C made up 52% of residue C.

The higher amounts of CdfR in the bulk soil of the chickpea rotations, relative to the other rotations ($P$>0.05) can be explained by the larger root biomass of the chickpea plants since there is a direct relationship between root biomass and rhizodeposition (De Graaf et al., 2007). This trend was not consistent in the HF, LF, VLF and WEOM as the P-W-P had greater amounts of CdfR in these soil fractions ($P$>0.05), despite the fact that the P-W-P soil had a smaller proportion of soil allocated to the HF, LF, VLF and WEOM than the CP-W-CP. It is possible that the pea rhizodeposits were more bioavailable to the SMB for use and incorporated into the SOM pools, than the chickpea rhizodeposits. Wichern et al. (2007a) reported that pea rhizodeposits were more labile and more quickly decomposed than canola rhizodeposits. A low C:N ratio for the pea rotation SMB-C rhizodeposits would support this theory since the lower C:N ratio of pulse crop residues result in more efficient conversion of residues to SOM (Lemke et al., 2007). Due to mass spectrometry analysis problems, SMB-C and SMB-$^{13}$C results were not produced and it is not possible to confirm this hypothesis.

The release of labile C from the roots can have a “microbial activation” effect where the decomposition of SOM is increased and the dissolution of insoluble minerals is accelerated by the soil microbial communities (Jones et al., 2004). Even minimal amounts of root exudates can enhance SOM turnover and increase the amount of plant available soil N (De Nobili et al., 2001). Although there were no statistical differences detected between crop rotations for the amount of CdfR in the bulk soil or soil pools ($P$>0.05), the CP-W-CP rotation tended to have more CdfR in the bulk soil than the other rotations. The CP-W-CP rotation also had the smallest $NO_3$ pool,

relative to the other rotations. The low allocation of $NO_3$ in the CP-W-CP may be a result of plant uptake as a result of "microbial activation" caused by a greater release of labile C from the roots.

Environmental and plant genetic and physiological factors can affect the flux of C from the roots to the soil as well as the size and structure of the root (Nguyen, 2003). Varying soil conditions and stress factors such as extreme moisture levels and mineral toxicities can alter the release of C from the roots and cause changes in root growth, structure and exudation (Jones et al., 2004). In the field, with unrestricted root growth there would likely be a greater root biomass, relative to this study (Wichern et al., 2008). The chickpea root biomass was greater than the pea and lentil root biomass ($P$=0.002); however this difference may not have existed in the field, where water stress is a threat. Approximately 90% of the world's chickpea is produced under rain fed conditions. Exposure to terminal drought stress throughout the reproduction stage has resulted in chickpea yield losses of 3.4 million ha (Sharma, 2004-2005). Likewise, water stress negatively affects lentil. Talukdar (2013) reported a reduction in lentil biomass, net phososynthetic rate and nodulation with periods of water stress.

Consistent with this study, previous studies have also reported a lower C:N ratio in the HF than in the LF and a higher C:N ratio in the HF than in the WEOM (Gregorich et al., 2006; Gregorich and Beare, 2007; Comeau, 2013). The %C and %N of the VLF are also consistent with Comeau (2013) in the lentil and pea phase of L-W-L and P-W-P rotations on a Brown and Dark Brown Chernozem; however the %C and %N of HF, LF and WEOM soil pools were 50% to 60% lower in this study than Comeau (2013). Based on the dynamic of captured residue C proposed by Comeau (2013), the majority of the crop residues in the soil in this study were freshly incorporated residues. Residue is first incorporated into the VLF and is then solubilized by the SMB and some partially decomposed plant material enters the LF. Additional decomposition allows the residues to be absorbed into the HF. This theory as well as the roll of lower C:N ratios allowing more efficient decomposition is supported by the tendency for lower C:N ratios in the (roots and rhizodeposits) of the CP rotations along with a larger VLF pool, higher VLF-C and higher VLF-CdfR trends in the CP rotations.

The majority of the C:N ratios of the rhizodeposits in each soil fraction are higher than the C:N ratio of the soil in each fraction, which suggests that the residues have not directly affected the soil C:N ratio. However, the lower C:N ratios of the rhizdeposits in the WEOM than the C:N ratios of the WEOM pool suggests that the added BG residues increased the soil C:N

ratio in the WEOM. Furthermore, Comeau et al. (2013) describes the HF, LF and VLF residues as always transferring C to the WEOM, therefore it is possible that the transfer of C from these pools is also raising the WEOM pool C:N ratio. The C:N ratio of the BG residues (the only added residues to the soil) here range from 5.1 to 55.8. This is not consistent with the C:N ratio range of 25 to 40 for pulse crops and 70 to 100 for cereals, reported by Stevenson and van Kessel (1996).

There was 0.7 to 1.37 g LF-C $kg^{-1}$ soil, 0.5 to 0.8 g LF-C $kg^{-1}$ soil and 0.4 g LF-C $kg^{-1}$ soil recovered in the chickpea rotations, pea rotations and the L-W-L rotation, respectively. The greater LF-C under the chickpea rotations does not completely agree with the literature, which states that relative to cereals, lentil and chickpea may reduce SOC levels as a result of less residue C production, while pea can maintain SOC levels similar to wheat due to residue C inputs (Lemke et al., 2007). However Campbell et al. (2001) reported comparable SOC levels in wheat-lentil and continuous-wheat systems, despite lower C inputs from the wheat-lentil rotation. The lower C inputs may have been counterbalanced by greater stabilization efficiency in the wheat-lentil rotation. In this study, it is likely a result of the larger chickpea plant aboveground biomass and root biomass contributing more C to the soil. This suggests that a longer time frame is required to further investigate the effects of crop rotation on SOC dynamics.

*4.5.1.2 Nitrogen*

Residue N in the chickpea, pea and lentil rotations was derived from a greater amount of BG residue N (roots and rhizodeposits) (61.8% to 81.5%) than ABG (pods, leaves and stems) residue N (18.5% to 38.2%). This compliments Arcand et al. (2013b), where 60.6% of pea residue N was from BG contributions. There were no differences between pulse crop rotations in the ABG and BG partitioning of N ($P>0.05$); however, there was a greater amount of residue N allocated to the roots of the chickpea rotations (2%) than there was in the lentil (0.7%) and pea (0.6%) rotations ($P<0.001$) (Fig 4.2b).

Lopez-Bullido et al. (2012) found 61% of total plant N was NdfR, in a wheat-chickpea rotation, and that 90% of BG (roots and rhizodeposition) plant N was NdfR. Similarly, Khan et al. (2003) reported BG-N as 68% of total plant N in chickpea. This is consistent with the wheat-chickpea rotations of this study. Nitrogen-dfR made up 62% and 66% of total plant N and 98% of BG (roots and rhizodeposition) plant N, in the two chickpea rotations preceded by wheat.

Based on the analysis of a compilation of legume (alfalfa, clover, lentil, pea and faba bean) BNF studies, lentil and pea rotations fix more N than faba bean rotations (Anglade et al., 2015). The same study also reported pulse crop BG-N ranged from 11% to 56% of total plant N, while NdfR was 1.4% to 51% of total plant N and in field pea, NdfR was as high as 98% of BG-N. These findings are consistent with the findings of this study as rhizodeposited N made up 46% of total plant N and 99% of total BG-N, in the pea rotations.

Gan et al. (2010) reported 14% of plant N was allocated to the roots at maturity in chickpea, lentil and pea; however this study found lower distributions of 1.5%, 0.4% and 0.4% of plant N allocated to the roots of chickpea, lentil and pea, respectively. Calculating BG-N is difficult because N rhizodeposits are not held in a specified area and fine roots are often too insubstantial to be collected by hand. The variation in the root collection technique used in this study compared to other studies may have contributed to variability in the root and rhizodeposition results. Although 100% of the roots were not collected from the soil in this study, the differences in root biomass between crop rotations are evident ($P$=0.002).

The greater chickpea root biomass relative to pea and lentil can explain the trend of greater amounts of NdfR in the bulk soil, the HF and the inorganic N pools of the chickpea rotations since the magnitude of rhizodeposition is proportional to root biomass (De Graaf et al., 2007). Nitrate-N has been previously reported as 13% of NdfR in non-fertilized pea (Arcand et al., 2013b). In this study, $NO_3$-NdfR made up only 2% of bulk NdfR. In chickpea, 90% of the SMB-N was NdfR, in lentil 98% of SMB-N was NdfR and in pea 99% of the SMB-N was NdfR. These proportions of SMB-NdfR are higher than the findings of Wichern et al. (2007b), where 18% of pea SMB-N was NdfR. The higher proportion of SMB-N derived from rhizodeposition in the pulse crop rotations in this study suggest that the rhizodeposits are of higher quality than in studies where a lesser proportion of SMB-N is derived from rhizodeposition (Wichern et al., 2007b). There is an inadequate amount of research on what factors (species, growth conditions, etc.) control the distribution of N to the roots and rhizodeposits (Anglade et al., 2015).

#### *4.5.1.3 Root:Shoot Ratio and Rhizodeposition*

The Legume Futures Report 1.5 (Baddeley et al., 2014) provides root:shoot ratios for chickpea, lentil and pea of 0.4, 0.4 and 0.1, respectively. These ratios are higher than those found

in this study, yet rhizodeposition of C and N is comparable to previous studies (Wichern et al., 2007a; Fustec et al., 2010). Again, the variability in root collection methods along with collecting only a fraction of the root biomass may have contributed to the lower root:shoot ratio here.

Mayer et al. (2003) suggest that in pot experiments the root-to-shoot ratio is in favour of the ABG biomass and not root biomass, relative to field experiments. Wichern et al. (2007a) found a higher root-to-shoot ratio in pea and oat plants resulted in greater amounts of rhizodeposition. The amount of CdfR and NdfR in the pulse crop rotations here is consistent with other studies, despite the lower root-to-shoot ratios reported in this study (Table 4.3). This opposes the theory that rhizodeposition is proportional to root biomass (De Graaf et al., 2007). It is possible that the energy and nutrients reserved for root growth in other plant studies has been allocated to rhizodeposition in this study; however, the altered root collection technique used here is the most likely explanation for the reduced root-to-shoot ratios reported by this study.

### 4.5.2 Aboveground and belowground partitioning of $^{13}C$ and $^{15}N$

#### *4.5.2.1 $^{13}C$*

The ABG and BG partitioning of $^{13}C$ did not differ between pulse crop rotations. A greater distribution of recovered $^{13}C$ in the ABG (pods, leaves and stems) residue (97.0% to 99.8%) than in the BG residue (0.2% to 3%) of all the rotations agrees with the findings of Wichern et al. (2007a) using pea. Wichern et al. (2007a) found a distribution of 17.6% BG-$^{13}C$ in pea, which is higher than the 0.1% and 0.3% BG $^{13}C$ recovered in the two pea rotations in this study. The heterogeneity of the $^{13}C$ label in the plant parts is common with repeat-pulse $^{13}C$ labeling and must be taken into account when reviewing $^{13}C$ residue transfer to soil (Sangster et al., 2010). The lower allocation of $^{13}C$ to the roots relative to ABG biomass in this study is consistent with the findings of other $^{13}CO_2$ labeling studies (Comeau et al., 2013; Matus et al., 2014). Using multiple injections of HCl, to produce multiple $CO_2$ spikes will also have contributed to the heterogeneity of the $^{13}C$ label distribution. The multiple injection technique causes continually increasing $^{13}C$ enrichment in the chambers, but was chosen to ensure the plants were exposed to adequate levels of $^{13}CO_2$.

A total of 43.1% and 45.1% of the $^{13}C$ applied to the pea plants was recovered in the plant soil system, which is higher than the 32.4% recovery of $^{13}C$ in the mature pea plant parts

and rhizodeposits in Wichern et al. (2007a). The majority of the ABG-$^{13}$C was allocated to seed in this study; however, Wichern et al. (2007a) found the stems and leaves held the greatest amount of $^{13}$C, followed by the seed. A greater amount of $^{13}$C was recovered in the roots of pea and lentil than in the rhizdeposits ($P$>0.05), which was also the trend for pea plants in Wichern et al. (2007); however the chickpea rotations did not follow this trend and allocated equal or greater amounts of $^{13}$C to rhizodeposition than the roots. Less than 100% recovery of $^{13}$C may be a result of gaseous $^{13}$C loss via $CO_2$ due to microbial respiration (Nguyen, 2003; Kuzyakov and Schneckenberger, 2004; Jones et al., 2009).

Due to technical issues with analysis of the SMB extracts it was not possible to recover any of the SMB-$^{13}$C. Continuous pulse labeling may have been more suitable here in order to increase the extent to which the SMB was enriched with $^{13}$C (Meharg, 1994). However, the successful enrichment of the SMB in chapter 3, as well as in other studies (Rangel-Castro et al., 2005; An et al., 2015) suggests that pulse labeling is effective enough to produce $^{13}$C-SMB results and the error in this case was in the analysis of the extracts by the mass spectrometer.

*4.5.2.2 $^{15}N$*

All of the pulse crop rotations contributed a greater proportion of $^{15}$N to ABG residues (70.4% to 97.7%) than to BG residues (2.3% to 29.6%). This trend is consistent with Arcand et al. (2013b), where 66% of residue $^{15}$N was allocated to the ABG pea residue. Recovery of $^{15}$N in the pea plants in Arcand et al. (2013b) ranged from 77% to 88%, which is similar to the range of $^{15}$N recovered in the plant-soil systems of chickpea, lentil and pea, in this study (50.4% to 87.3%). The greater allocation of residue $^{15}$N to the roots of the chickpea rotations than in the L-W-L and P-W-P rotations is likely a result of the greater root biomass in the chickpea and P-M-P rotations; however the distribution of residue $^{15}$N to the roots of P-M-P was also greater than in the lentil and P-W-P rotations, despite having a lower root biomass. Belowground transfer of residue $^{15}$N to the roots in the P-M-P appears to be stronger than in the lentil and P-W-P rotations.

Less than 100% recovery of $^{15}$N may be a result of gaseous $^{15}$N loss via denitrification and volatilization of $^{15}NH_3$ from the leaves, as well as residual $^{15}$N being held in the stem wick apparatus (Zebarth et al., 1991; Mayer et al. 2003). Experimental errors in quantifying plant part $^{15}$N to calculate the total $^{15}$N balance may have also resulted in incomplete $^{15}$N recovery (Mayer et al., 2003). Preferential $^{15}$N enrichment of the ABG plant parts over the roots, as well as the

heterogeneous distribution of $^{15}N$ residue, is consistent with other studies using shoot labeling techniques (Russell and Fillery, 1996; Wichern et al., 2007a,b; Arcand et al., 2013). The lower distribution of $^{15}N$ to the roots is likely a result of applying the $^{15}N$-urea directly to the plant stem (Wichern et al., 2010). One of the limitations of using $^{15}N$ shoot labeling techniques to follow ABG and BG residue N to the soil is the uneven distribution of $^{15}N$ to the ABG and BG plant parts. Furthermore, errors in the NdfR estimation are probable as a result of heterogeneous distribution of $^{15}N$ within the plant roots.

The assumption that $^{15}N$ enrichment of all recovered roots is representative of the enrichment of NdfR was not fully met here due to the probability of incomplete recovery of fine roots, the heterogeneity of $^{15}N$ within the roots, as well as not taking root nodulation into consideration (Khan et al., 2002).

**4.6 Conclusion**

This experiment examined the partitioning of C and N to the plants and soil in cereal-pulse crop rotations. The stem-wick $^{15}N$ labeling method, the repeat pulse $^{13}C$ labeling method, density fractionation and SMB and inorganic N extraction revealed: 1) ABG residue C (pods, leaves and stems) was greater than BG (roots and rhizodeposits) residue C in all pulse crop rotations except P-M-CP and P-W-CP; 2) the BG residue N was greater than ABG residue N (pods, leaves and stems) in all of the pulse crop rotations; 3) the majority of BG residue N and BG residue C was allocated to rhizodeposition; 4) the ABG and BG contributions of C or N to the soil did not differ between the pulse crop rotations.

The partitioning of residue C in the plant parts and rhizodeposits did not differ between pulse crop rotations. The residue N in the plant parts and rhizodeposits did not differ between pulse crop rotations, except for in the roots, where root N of the chickpea rotations exceeded that of lentil and pea. The majority of residue $^{13}C$ and $^{15}N$ was allocated to ABG residue and not BG residue. The residue $^{13}C$ in the plant parts and rhizodeposits did not differ between pulse crop rotations. The allocation of residue $^{15}N$ did not differ between pulse crop rotations, except for in the roots and total BG-$^{15}N$. The allocation of residue $^{15}N$ to the roots was greater in the P-M-CP rotation than the L-W-L and P-M-P rotations.

The soil C and N did not differ between pulse crop rotations in the bulk soil, HF, LF, VLF, SMB or inorganic N pools. However, the soil $^{13}C$ differed amongst crop rotations in the

LF. The LF-$^{13}C$ in the CP-W-CP exceeded that of the lentil and pea. The soil $^{15}N$ was greater in the pea rotations in the WEOM than the chickpea and lentil rotations. In the $NH_4$ pool the CP-W-CP rotation soil $^{15}N$ was greater than pea and lentil. The amount and distribution of CdfR and NdfR did not vary significantly between pulse crop rotations in any of the soil pools. This study drew similarities and differences to other studies that measured rhizodeposition in legumes.

# 5. SYNTHESIS AND CONCLUSIONS

The number of rhizodeposition studies reported in the literature has increased over the past few decades; however this research has focused primarily on the rhizodeposition of C (Nguyen, 2003; Jones et al., 2009). The simultaneous quantification of C and N derived from rhizodeposition will improve our understanding of the ABG and BG partitioning of C and N by plants, as individual nutrients and also as coupled nutrients dependent upon one another (Wichern et al., 2007a,b). Yet there are few rhizodeposition studies that have produced simultaneous C and N quantification. The research presented in this thesis aids in the understanding of the effects of pulse crop (chickpea, lentil and pea) sequencing on the partitioning of C and N.

The overall goal of this research was to determine the effect of pulse crop rotation on the partitioning of ABG and BG inputs of C and N to soils, and to examine the contributions of rhizodeposited C and N to various SOM pools, the inorganic N pool and the SMB. The plants were labeled with the stable isotopes $^{13}C$ and $^{15}N$ to quantify C and N derived from rhizodeposition.

## 5.1 Summary of Experimental Findings

The ability of chickpea, lentil, and pea to biologically fix atmospheric $N_2$ makes the inclusion of these three pulse crops in rotation with non-pulse crops essential in Saskatchewan cropping systems to help reduce overall N fertilizer use. Furthermore, chickpea lentil and pea are desirable SOM builders due to their additions of C to the soil via roots and rhizodeposits, as well as from low C:N ratio, high quality crop residues.

Repeat pulse $^{15}N$ and $^{13}C$ labeling revealed that chickpea, lentil and pea in rotation with mustard or wheat provide greater amounts of CdfR and NdfR to the bulk soil, than continuously grown chickpea, lentil and pea (Fig. 5.1). The CdfR and NdfR in the VLF pool were also greater in chickpea, lentil and pea in rotation with mustard or wheat than in the continuous chickpea, lentil and pea systems (Tables 5.1 & 5.2).

Rhizodeposition was greater in the non-continuous pulse crops despite the root:shoot ratio in the continuous pulse crops exceeding that of the non-continuous pulse crops. The allocation of plant biomass to the roots was greater in the continuous chickpea plants (15%) than in the chickpea grown in rotation with non-pulse crops (7%). Gill et al. (2009) reported negative effects on root growth of chickpea intercropped with wheat due to the allelopathic effect of wheat root exudates. A factor causing the lower CdfR and NdfR under continuous chickpea, lentil and pea is the greater availability of previously planted BG pulse crop residue to the soil microbial communities for mineralization (Knight, 2012). When a non-pulse crop precedes a pulse crop, the residues left in the soil are not immediately available for mineralization and the subsequently planted pulse crop can further contribute to the residual soil C and N stores via rhizodeposition. This agrees with Arcand et al. (2014) where BG-N residue under lentil was more readily mineralized than BG-N residue under wheat.

There was a trend towards a higher allocation of total plant N to the $NO_3$-NdfR pool in the continuous pulse crop systems (17.5% to 25.3%) than in the non-continuous pulse crop rotations (0.3% to 3%). There was also a greater allocation of total plant C to bulk CdfR in the non-continuous pulse crop rotations than in the continuous chickpea, lentil and pea. This may be explained by root exudates acting as primers to promote SOM turnover and increase N availability (De Nobili et al., 2001; Jones et al., 2004; Cheng et al., 2014; Haichar et al., 2014). It is possible that $NO_3$-NdfR in the pulse crop rotations was taken up by the plants at a greater rate than the continuous pulse crops, due to a greater CdfR pool. However, there was no difference in the total $NO_3$ soil pools between the continuous and non-continuous pulse crop systems, which suggests that uptake of N by the plants was primarily from the more readily available $NO_3$-NdfR (Wichern et al., 2007b).

The chickpea, lentil and pea in rotation with mustard or wheat also had a greater proportion of SMB-N derived from rhizodeposition than the continuous chickpea, lentil and pea. This agrees with Wichern et al. (2007b), where the proportion of SMB-N derived from rhizodeposition in non-continuous pea was higher than in wheat. This finding confirms that the quality of rhizodeposits differs with plant rotations and causes a lower availability of rhizodeposits to the SMB in the continuous pulse crop rotations. The greater availability of the rhizodeposits to the SMB in the pulse crop rotations relative to the continuous pulse crops is

explained by the lower C:N ratio of the rhizodeposits in the non-continuous chickpea, lentil and pea (Table 5.3).

The dual labeling of plants with $^{13}C$ and $^{15}N$ was a unique feature of this project; however when comparing plant and soil C and N levels between experiments there are several factors that must be considered. It is important to note that the plants in the second experiment (Chapter 4) had higher biomass, which caused a greater C and N content, relative to the first experiment (Chapter 3). However, the percentage allocation of biomass to the ABG plant parts did not differ between the two experiments. It is possible that the crop sequencing contributed to larger, healthier plants in the second experiment, relative to the smaller plants in the first experiment where pulse crops were grown three years in a row. Regardless of the cause, the difference in plant biomass between the two experiments will have resulted in the higher levels of C and N in the ABG plant parts as well as in the BG residue recovered in the soil. Furthermore, the $^{13}C$ and $^{15}N$ labeling period of the plants in the second experiment exceeded the labeling period of the plants in the first experiment by two weeks. The longer labeling period for the non-continuous plants (Chapter 4) occurred because the plants were larger and stronger a week earlier in their growth period and thus ready for labeling a week earlier than the continuous plants in Chapter 3. The non-continuous plants (Chapter 4) also grew for one week longer before reaching maturity than the continuous plants (Chapter 3) and were therefore labeled for a week longer than the plants in Chapter 4. The higher doses of $^{13}C$ and $^{15}N$ applied to the plants in the second experiment (Chapter 4) along with the greater biomass of the plants will have contributed to the greater BG additions of C and N to the soil.

Along with the difference in the amount of C and N recovered in plant residue, the distribution of plant C and N differed between the two experiments. In the first experiment, C was primarily allocated to rhizodeposition, except for the L-W-L sequence, where it was primarily allocated to stems. In the second experiment however, the seed contained the most C in chickpea and pea, and again lentil contained the most C in the stems. It was not possible to compare the residue C in the SMB of the continuous pulse crops (Chapter 3) versus that of the non-continuous pulse crops (Chapter 4) due to issues tracking $^{13}C$ into the SMB of the non-continuous pulse crops. This would have been a useful indicator of the difference in rates of residue decomposition and changes in SOM content between the continuous and non-continuous pulse crops. The distribution of plant N amongst ABG plant parts was similar between the two

experiments; however there was a greater allocation of plant N to BG components in the second experiment (Chapter 4).

Patterns of distribution of plant and soil C and N in the soil pools were similar between both experiments (HF>LF>VLF). However, there was more plant and soil N allocated to the SMB in the second experiment (Chapter 4) than in the first experiment (Chapter 3). There was a similar pattern of recovery of $^{13}C$ and $^{15}N$ in the soil pools, between the two experiments. There was however a higher percentage of recovery in the second experiment than in the first, which is a result of more $^{13}C$ and $^{15}N$ applied in the second experiment. The amount and distributions of CdfR and NdfR in the soil pools of the second experiment exceeded those of the first experiment, apart from the greater allocation of plant and soil NdfR to the $NO_3$ pool in the first experiment, than there was in the second experiment.

In both studies the C:N ratio of the rhizodeposits in the LF and VLF was greater than the C:N ratio of the SOM in the LF and VLF, apart from the continuously grown pea in the first experiment. The higher C:N ratio of the rhizodeposits than of the SOM pools suggests that the legumes grown in this study provide a greater amount of CdfR to the soil, relative to the amount of NdfR and that the CdfR is subject to relatively slow biodegradation (Balesdent and Balabane, 1996). However, the distribution of plant C and N to rhizodeposition (Tables 3.5, 3.7, 4.5 & 4.7) show that 23 to 30% and 40 to 66% of plant N was allocated to rhizodeposition in the continuous (three year) and non-continuous pulse crops, respectively. While, 11 to 16% and 16 to 40% of plant C was allocated to rhizodeposition in the continuous (three year) and non-continuous pulse crops, respectively. The high additions of NdfR to the soil via rhizodeposition may have resulted in high immobilization of NdfR by the soil microbial population causing high CdfR:NdfR ratio. There are difficulties associated with predicting the evolution of C and N through out plant residue decomposition and problems with comparing existing data to other types of residues and to different soils with varying levels of native soil N (Mary et al., 1996).

It is also possible that the residual CdfR in the soil from the previously grown crop was mineralized immediately causing low mineralizable C to remain. Lu et al. (2003) found that the rhizodeposited C from previously grown rice was mineralized rapidly at first and then slowly. A higher proportion of N would enhance the nitrifiers and denitrifiers and result in an increase in the CdfR:NdfR ratio, as nitrifiers and denitrifiers use inorganic N as an energy source (Arp and Stein, 2003). Furthermore, there were no ABG crop residues added to the soil in both studies

conducted here. Perhaps the lack of ABG crop residues added to the soil meant there was no spike in microbial metabolism and no take over by the r-strategist microbes (Bastian et al., 2009). R-strategists are opportunistic microbes that dominate soils after fresh residues are incorporated. The r-strategists consume soluble and simple substrates and grow rapidly. The majority of available substrates are incorporated by r-strategists before the slower growing K-strategist microbes become dominant (Fontaine et al., 2003). Had ABG residues been added a larger increase in $CO_2$ emissions from the soil microbial communities and increase in amino acids in the soil as decomposition progressed may have caused a lower CdfR:NdfR ratio in the VLF and LF, which was not the case here.

The only soil pool that had a greater C:N ratio than the CdfR;NdfR ratio was the WEOM. Lu et al. (2003) found that the most rapid decrease in rhizodeposited C was in the WEOM pool. A rapid initial decrease in WEOM-CdfR along with the transfer of some water soluble compounds from the WEOM to the HF may have contributed to the lower CdfR:NdfR ratio relative to the C:N ratio of the WEOM (Comeau, 2012).

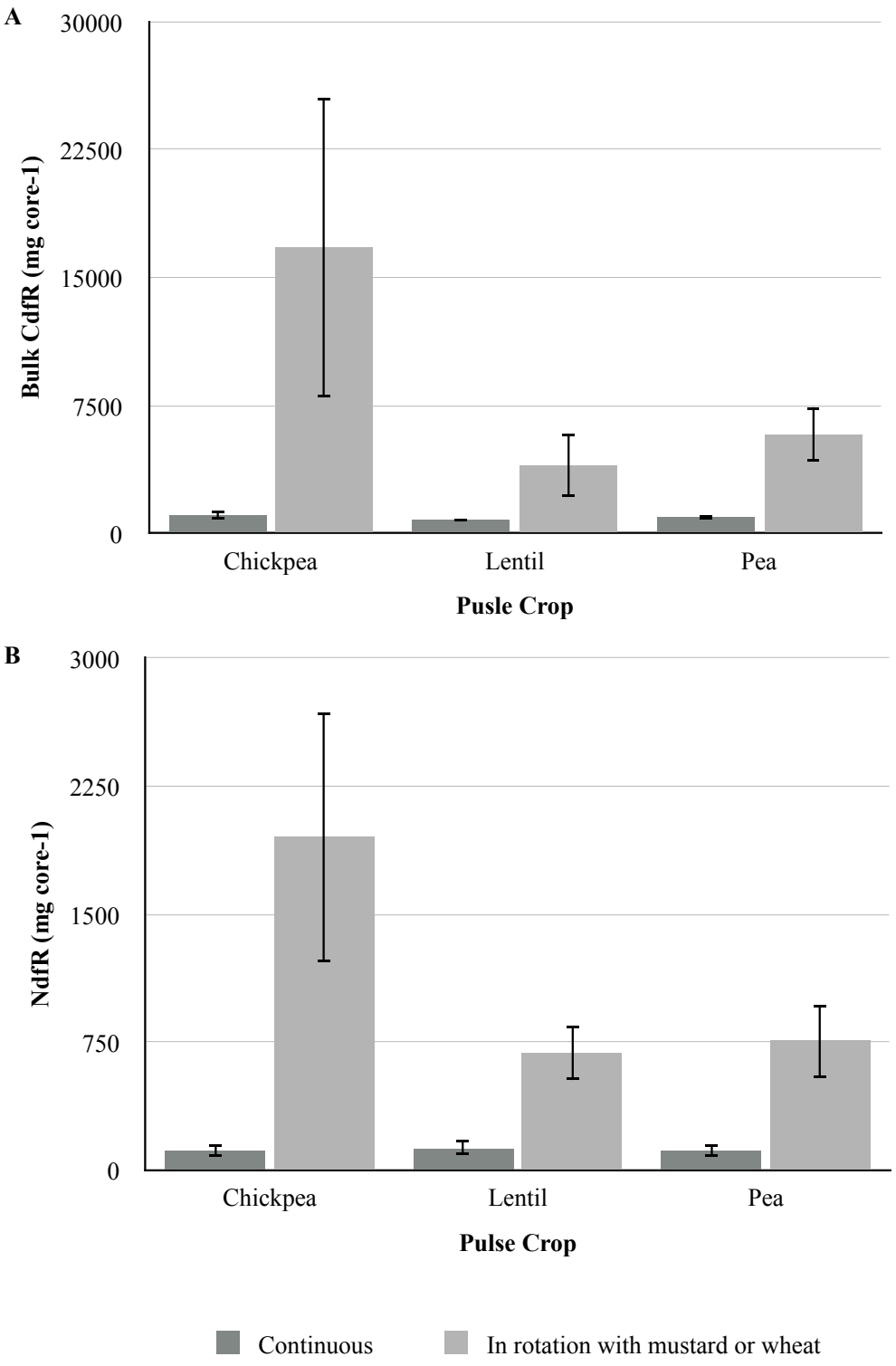


**Figure 5.1. Bulk CdfR (A) and bulk NdfR (B) under continuous and non-continuous chickpea, lentil and pea grown in a greenhouse and supplied with $^{15}N$ and $^{13}C$ using the stem-wick method and atmospheric $^{13}C$ labeling, respectively. Values are means of four cores and (Standard errors).**

**Table 5.1. The VLF-CdfR (mg core$^{-1}$) in continuous and non-continuous chickpea, lentil and pea rotations grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of three or four cores and (Standard errors).**

| | VLF-CdfR (mg core$^{-1}$) | |
|---|---|---|
| | **Continuous Pulse Crops (Ch. 3)** | **Non-Continuous Pulse Crops (Ch. 4)** |
| **Chickpea** | 1.4 (0.003) | 368.6 (96.28) |
| **Lentil** | 0.8 (0.003) | 182.1 (91.74) |
| **Pea** | 0.6 (0.008) | 246.9 (210.97) |
| ***P value*** | 0.7 | 0.9 |

† Same letters following means ± standard errors (n=4) indicate no significant difference between treatments within each plant part ($p$>0.05) according to Tukey's HSD test.

**Table 5.2. The VLF-NdfR (mg core$^{-1}$) in continuous and non-continuous chickpea, lentil and pea rotations grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of three or four cores and (Standard errors).**

| | VLF-NdfR (mg core$^{-1}$) | |
|---|---|---|
| | **Continuous Pulse Crops (Ch. 3)** | **Non-Continuous Pulse Crops (Ch. 4)** |
| **Chickpea** | 0.04 (0.014) | 13.2 (8.58) |
| **Lentil** | 0.03 (0.012) | 10.6 (4.23) |
| **Pea** | 0.04 (0.024) | 15.5 (10.22) |
| ***P value*** | 0.8 | 0.6 |

**Table 5.3. The C:N ratio of the bulk rhizodeposits in continuous (Three year) and non-continuous chickpea, lentil and pea rotations grown in a greenhouse and labeled with $^{15}$N-urea and $^{13}CO_2$. Values are means of three or four cores and (Standard errors).**

| | C:N Ratio | |
|---|---|---|
| | **Continuous Pulse Crops (Ch. 3)** | **Non-Continuous Pulse Crops (Ch. 4)** |
| **Chickpea** | 12.3 (4.65) | 8.7 (3.38) |
| **Lentil** | 7.6 (2.26) | 5.5 (1.36) |
| **Pea** | 26.9 (22.19) | 9.2 (2.95) |
| ***P value*** | 0.9 | 0.7 |

## 5.2 Future Research

Future studies investigating the effects of continuous pulse crop systems on the above and belowground partitioning of C and N should consider quantifying BNF by the continuous and non-continuous pulse crop rotations and the relation to the amount of rhizodeposition taking place. Despite the difficulties associated with determining the N provided to subsequent crops (Walley et al., 2007), the factors influencing whether root derived C and N are directly utilized

by subsequent crops or whether it is incorporated in the SOM is also relative to future research.

Future studies investigating the effects of pulse crop rotations on the ABG and BG partitioning of C and N should continue to quantify the BG contributions of C and N to soils, using stable isotope techniques. The percent N derived from the atmosphere by the rotations should be quantified at the same time as the quantification of BG-C and N contributions in order to better understand the relationship between BG-C and BG-N cycling, BNF and balancing N inputs and outputs. Investigation of these factors in a long-term pulse crop rotation system would help build on the research completed here. A long-term pulse crop study would be beneficial to better understand the behaviour of BG-C and BG-N dynamics beyond a three-year rotation. Previous long-term studies using the stem wick method have demonstrated an increase in rhizodeposition of N as plant age and plant N content increase; however there is little information on the effects of N partitioning by plants (Fustec et al., 2010).

The growing conditions in this study were intended to replicate field conditions as much as possible; however, quantifying BG-C and BG-N contributions to the soil with long-term rotations in the field is recommended. There are differences between plant root:shoot ratios in the field relative to those in pots (Poorter et al., 2012), and the relationship between plant root biomass and rhizodeposition has been highlighted (De Graaf et al., 2007). Comparative labeling studies that investigate the partitioning of C and N at various $^{13}C$ and $^{15}N$ labeling frequencies and durations should also be conducted to better understand the influence of labeling techniques and practices.

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